"A STUDY ON MYCOLOGICAL PROFILE OF ONYCHOMYCOSIS IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN''

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON MYCOLOGICAL PROFILE OF ONYCHOMYCOSIS IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN"** is a bonafide record of work done by **DR.A.GOMATHI CHITRA**, during the period of her Post Graduate study from 2012 to 2015 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfillment of the requirement of **M.D MICROBIOLOGY** degree Examination of The Tamilnadu Dr. M.G.R Medical University to be held in April 2015.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON MYCOLOGICAL PROFILE OF ONYCHOMYCOSIS IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **October 2013 – September 2014** under the guidance of **Dr.S.Vasanthi, M.D.,** Professor, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical Unversity, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2015.

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TURNITIN ANTI-PLAGIARISM SOFTWARE

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ABSTRACT

A STUDY ON MYCOLOGICAL PROFILE OF ONYCHOMYCOSIS IN DIABETIC PATIENTS IN A TERTIARY CARE HOSPITAL AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN

INTRODUCTION: Onychomycosis among diabetics are very common and increases the risk of developing secondary bacterial infection and cellulitis if untreated.

AIMS AND OBJECTIVES: To identify the etiological fungal pathogens in onychomycosis among diabetic patients.To determine the epidemiological profile of pathogens and to study the antifungal susceptibility pattern of the isolates.

MATERIALS AND METHODS : The study was carried out in the Institute of Microbiology in association with Department of Dermatology and Institute of Diabetology,Madras Medical College,RGGGH, Chennai during October 2013 to September 2014.Nail scrapings were collected from all known diabetic patients above the age of 18 years who were diagnosed with onychomycosis irrespective of their glycemic status.The samples were subjected to direct examination and culture.The fungal pathogens were identified and their antifungal susceptibility pattern was done.

RESULTS:The culture and KOH positivity rate was 46.28% and 70.29% respectively.*Candida species* was the most common isolate (39.50%) followed by non-dermatophytic moulds (34.58%) and dermatophytes(12%).*Candida parapsilosis*(75%) was the most common etiological agent among *Candida* species.Among non-dermatophytic moulds,*Fusarium* species was the most etiological agent.*Candida* species were common among females in finger nail infection. All the *Candida* species were sensitive to azole drugs.Voriconazole was found to have lower MIC range in non-dermatophytic moulds except *Fusarium* species.Terbinafine was found to have lower MIC range in case of dermatophytes.

CONCLUSION:

This study highligts the role of *C.parapsilosis* as a emerging pathogen in diabetic patients and fluconazole still remains as an effective antifungal agent among the *Candida* species.Non-dermatophytic moulds should not be merely considered as contaminants and the diagnosis should be confirmed by repeated sampling.

INTRODUCTION

Onychomycosis(OM) is a term which encompasses chronic fungal infection of the nail plate . OM can be caused by dermatophytes, yeasts or moulds.Onychomycosis accounts for about 20 - 40% of nail disorders and 30% of mycotic cutaneous infections[7].Until the 1990s OM was not much discussed. But nowadays, OM are considered as a major source of interest and being studied in various epidemiological regions.

Prevalence of onychomycosis is about 0.5% to 5% in the general population[28] .But the prevalence of OM depends on number of factors such as age, socio-economic condition , occupation, climatic conditions and the immune status of the patient.

The worldwide prevalence of diabetes mellitus(DM) has increased in recent years. Individuals with DM acquire infection with a greater frequency and severity, as they often have neuropathy or arterial insufficiency[96]. Fungal infections constitute the most common type of infection among diabetics. One of the predictors for diabetic foot syndrome is OM[9] . Diabetic patients are atleast twice as likely to suffer from onychomycosis than normal individuals. In diabetic individuals, the prevalence of OM is about 22%[12].

Diabetic individuals with onychomycosis are more prone for gangrene and foot ulcer when compared to those DM individuals without onychomycosis.The risk of lower leg amputation may be increased if fissures or traumatic ulcerations are followed by secondary infections. Thus nail infections represent a risk factor in diabetic patients because of possible sequels[9].

The clinical presentation may vary from nail discolouration, onycholysis of nail plate, hyperkeratosis to destruction of nail plate. As nail plate will be destroyed in many other nail disorders, it is necessary to diagnose OM correctly.

Onychomycosis, is caused by three groups of fungi, namely dermatophytes, yeasts and non-dermatophytic moulds, with the majority of onychomycosis caused by dermatophytes[96].

The non-dermatophytic moulds are saprophytic fungi, which can cause opportunistic infections in immunocompromised patients. However, their isolation from nails is becoming more frequent though they were intially considered as contaminants. But nowdays, these non-dermatophytic moulds are considered to be emerging pathogens.

Though the treatment of OM in diabetic patients is same as that of the patients without diabetes, treatment is more difficult in diabetic individuals because it requires long term therapy. Elderly diabetic patients require a longer duration to treat because the nails may grow slower[89] . Onychomycosis in diabetics may be associated with complications like cellulitis, diabetic foot ulcers and gangrene ,which will increase the admission rates in hospital and surgical interventions.

Hence early diagnosis and appropriate medical management of OM among diabetics using oral antifungals will be more cost effective than treating the complications[89].

The choice of antifungal agent is different in dermatophyte, non-dermatophytes and *Candida* infections causing OM. Antifungal susceptibility testing

is important because of emerging drug resistance.Antifungal drugs should ideally be given on the basis of *in vitro* sensitivity of the isolate.

Therefore, this study was undertaken to know the mycological profile among diabetic patients, their epidemiological profile and anti-fungal susceptibility patterns.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Nails are considered as one of the important component of human body, because the nail serves some important physiological and social functions. About 10% of dermatological conditions are due to nail disorders. Both physical and social disability can be caused by nail disorders.So it should never be underestimated or disregarded. Nail disorders are associated with number of heritable and non-heritetable syndrome. Nail unit may show specific changes which serves as a marker for wide range of systemic disorders. So nail has to be examined to complete the physical examination.[1]

ANATOMY OF NAIL:

The nail unit is composed of nail matrix and nail bed epithelium(generative portion), nail plate (product), nail folds and grooves (its framing), cuticles, eponychium and hyponychium(its sheathing), and fibrocollagen (supportive tissues). The finger nail grows at a rate of 0.1mm per day in young adults.Toe nails grows at a slower rate when compared to finger nails.The nail growth is even slower in patients with peripheral vascular disease[92].

FUNCTIONS OF NAIL:[1]

- Protection – Protects the terminal phalanx and finger tips
- Auguments the sensation of touch
- Permits scratching
- Manual dexterity - Gives precision in picking up small objects
- Utmost cosmetic importance.

HISTORICAL ASPECTS:[2]

In 1829, Parisian empiric Mahon was the first to describe the mycotic infection of nail. George Meissner was the first to observe the hyphal elements in Potassium hydroxide mount preparation from the nail sample in 1853 from an 80 year old male patient. The term ONYCHOMYCOSIS was first coined by Virchow in 1856.

The potassium suchole was the first antifungal agent used in 1903, followed by Whitfield's ointment. In 1904, Dubendorfer was the first to report *Candida albicans* infection in nail sample.

In 1910[3] , A book "Les Teignes" was published by Raymond Sabouraud which includes the classification of Dermatophytes into 3 genera,*Trichophyton*, *Microsporum* and *Epidermophyton* along with the genus *Acharion*.

The term "Leukonychia Trichophytica" was coined by Jessner for Superficial Onychomycosis in 1922. In 1926,the term "Leuconychia Mycotia" was coined by Rost for Superficial Onychomycosis.

In 1933,GaLitles and Evan described the nail infection due to *Hendersonula toruloidea.* Berseton and Keil was the first to prove that *Aspergillus* species can lead to OM in 1941.In 1948,Moore and Weises in USA reported the additional cases of OM due to *Aspergillus*.

Zaias in 1972[4], Classified the fungal nail infection into Distal and lateral subungual onychomycosis, Superficial onychomycosis and Proximal Subungual onychomycosis.

In 2011[5] ,a new classification was proposed by Baran along with Distal and lateral subungual onychomycosis, Superficial onychomycosis, Proximal subungual onychomycosis,this classification also includes Endonyx onychomycosis ,Total Dystrophic onychomycosis ,Mixed onychomycosis and secondary onychomycosis .

DEFINITIONS[6]

ONYCHOMYCOSIS :

A term which encompass the fungal infection of nail, caused by dermatophytes, non dermatophytic moulds, or yeasts.

Onychomycosis	-	Derived from the Greek word
"Onyx"	-	a nail
"mykes"	-	a fungus.

TINEA UNGUIUM :

Fungal infection of nail by dermatophytes.

UNGUAL ONYXIS :

A term which denotes the fungal infection of nail by yeast-like fungi .

UNGUAL CANDIDIASIS :

Denotes the fungal infection of nail by the genus *Candida*.

UNGUAL MYCOSIS :

A term used to denote the fungal infection of nail if the etiological agent is an opportunistic filamentous fungi.

EPIDEMIOLOGY

Onychomycosis(OM) is the most common infection. Onychomycosis accounts for about 20-40% of nail disorders and 30% of mycotic cutaneous infections. [7]

DIABETES MELLITUS

The worldwide prevalence of diabetes mellitus(DM) has been increased. Individuals with DM will acquire the infection with greater frequency and severity. In DM patients increased frequency of infection is due to incompletely defined abnormalities in cell mediated immunity and phagocytic function associated with hyperglycemia and also due to diminished vascularisation.[8]

DM patients have a chance of 2.5 to 2.8 times more to acquire onychomycosis than the individuals without DM. OM can result in development of foot ulcers because of the sharp, thick,brittle nail piercing the skin and also due to the vascular compromise.Vascular compromise is due to the increased subungal pressure because of the enlarged dystrophic nails.One of the serious complication of DM is diabetic foot ulcer. Foot ulcers can often lead to foot amputations and requires hospitalisation among them. One of the significant predictor for foot ulcer is OM. In OM, as a result of injury many pathogens will enter,which will lead to the further complications like osteomyelitis,cellulitis, gangrene and lower limb amputation[9]. Majority of the lower limb amputations are among DM patients[89].

The patients with diabetes and OM have a significantly higher rate of foot ulceration, gangrene than those diabetic patients without OM[10].

NAIL CHANGES ASSOCIATED WITH DIABETES MELLITUS[1]

INFECTION	VASCULAR LESIONS	NEUROPATHY	MISCELLANEOUS
Paronychia (Acute and Chronic) Onychomycosis Onycholysis	Beau's line Onychauxis Pterygium unguis Pterygium inversum unguis	Onychauxis Onychocryptosis Neurological dystrophy Reflex sympathetic dystrophy	Rosenau's depression Onychogryphosis Pincernail deformity Onychomadesis Leukonychia

PREVALENCE OF ONYCHOMYCOSIS AMONG DIABETICS

The prevalence of OM depends on many factors such as age, socioeconomic conditions, occupation, immune status of the individual, climate, environment and frequency of travel[11].

AUTHOR	PREVALENCE
Saunte DM *et al*..,[12]	22%
Levy LA *et al*..,[13]	22%
Dogra *S et al*..,[14]	22%
Gupta AK *et al*[15]	22%
Aditya K.Gupta *et al*..,[16]	6.5%

There is a positive correlation between OM and age and the severity of nail changes, but there was no correlation between OM and gender, type of DM[12].

The relationship between the duration of diabetes and the prevalence of onychomycosis is controversial[94][16] .

The mean age of the patients with OM was same as that of the patients with and without DM. The prevalance of OM was higher among the DM patients who had a weaker control of disease[95].

In 2003 in the United States,the total annual cost for toe,foot and leg amputations was almost $2 billion.These costs covered 112,551 of total amputations, an average cost for each procedure is $16,826.The total cost of amputations in 2001 among diabetic patients was more than $1.6 billion[89].

RISK FACTORS OF ONYCHOMYCOSIS:

AGE AND GENDER:

The prevalence of OM in children is very low (approximately 0·4%) . This may be due to reduced exposure to infected environment (communal showers, public changing rooms and saprophytic fungi), smaller nail surface area, faster nail growth and lower prevalence of tinea pedis and nail injuries [17].The disease is more common among elderly. Approximately, 10% of general population,20% of people above the age of 60 years,50% of people above the age 70 years and one third of DM individuals will have OM.[17]

OM is more common among males when compared to females.The gender difference may be due to the hormonal level differences (progesterone and other related hormones).[17]

GENETICS

OM is more common among certain family members suggesting the possibility of intrafamilial transmission[20]

An autosomal dominant pattern of inheritance in *Trichophyton rubrum* infection was reported.[20]

A case-control study was conducted in Mexican Mestizos to know the association of HLA class II with OM. In that study the patient with culture positive for *T.rubrum* infection along with controls are taken. OM was investigated for the first degree relatives of the patient. By polymerase chain reaction HLA typing was done in case and control samples.This study suggests that HLA-DR6 confers resistance against *T.rubrum* infection.[21]

The prevalence of tinea unguium was higher among the childrens with Down syndrome.[6]

SMOKING :[22]

There is a strong association between the OM and smoking history.

ENVIRONMENTAL FACTORS :[17]

Higher prevalence of OM was observed in the individuals who reside in urban areas.This may be due to 'urbanization' which is associated with many predisposing factors , such as overcrowding, communal bathing areas, and clothing habits, geographical and climatic differences between communities. Tinea pedis is more common individuals who wears shoe. A moist warm environment is produced within shoe which is ideal for fungal growth.[53]

SPORTS [17]

The prevalence of OM was reported to be two fold higher among atheletes when compared to non-atheletes.

FREQUENT NAIL TRAUMA :[6]

In females, trauma to the nail plate is usually due to continued exposure to detergents and water. Agricultural workers, cleaning employees, construction or iron and steel workers are more prone to micro traumas. Nail-biting or finger sucking, are common among childrens which may lead to nail trauma.

IMMUNODEFICIENCY :

HIV infected individuals are at greater risk of acquiring T.unguium and it correlates with CD4 count. PSO is more among them.The frequency of infection also more among the patients with lower CD counts(450cells /μL).[24]

HAEMODIALYSIS PATIENTS[25]

A Brazilian study reported the prevalence of OM as 39%. The most common isolate was dermatophye(69.23%) followed by *Candida* species(15.38%) and non-dermatophytic moulds(15.38%).In DM patient the risk of OM increses by 88% when compared to non- diabetic individuals on hemodialysis.This study concludes the higher frequency of OM among diabetics.

PSORIASIS:

The prevalence of OM depends on the duration and type of psoriasis.The probability of acquiring non-dermatophytic moulds is higher among psoriatics. The prevalence of OM was found to be high among psoriatic patients[26] [27].

PERIPHERAL VASCULAR DISEASE:

The prevalence of onychomycosis among the individuals with peripheral vascular disease was found to be 36%. *T. rubrum* was the most common pathogen. Increased prevalence of OM among individuals with peripheral vascular disease may be partly due to elderly and diabetic patients.

CONCOMITANT DERMATOPHYTE INFECTIONS[36]

Jacek C.Szepietowski *et al* conducted a study to know the prevalence of concomitant dermatomycoses in patients with OM .42.8% have concomitant fungal skin infections.The most common among them is T.pedis(33.8%) . Other concomitant dermatomycoses were T. cruris(4.2%) , T. corporis (2.1%), T.manuum (1.6%), and T.capitis (0.5%).There presence also depends on duration of OM.

CONSEQUENCES OF ONYCHOMYCOSIS :[37]

OM will impair the patient quality of life and it is more than a cosmetic inconvenience.

Physiological consequences :

1. Exacerbates the diabetic foot and can precipitate recurrent thrombophlebitis and cellulitis.
2. May trigger the bacterial infections, pain, dermatophytic reactions.

Psycho-social consequence: Loss of self esteem, loss of confidence, anxiety and depression.

Social effects: Impaired relationships,Produces fear of contagion.

It can also lead to economical burden.

CLASSIFICATION OF ONYCHOMYCOSIS:

Initially **Zaias** classified the OM into 3 distinct forms: distal and lateral subungual onychomycosis (DLSO), superficial white onychomycosis and proximal subungual onychomycosis (PSO).

A newer classification of OM was proposed by **Baran** et al.., as follows

1)DLSO 2) SWO 3) PSO 4)EO 5)TDO 6)MO 7)SO 8) CO

1) DISTAL AND LATERAL SUBUNGUAL ONYCHOMYCOSIS[DLSO]

This type of OM is the most common form (90%) and the most common agent is *T.rubrum*. It present as a patch of discolouration,yellow or white near the free edge of nail plate(lateral fold). Occasionally it may become black or dark brown. Nail plate will be lifted and cracks may appear because of the accumulation of soft subungual hyperkeratosis. Usually this type of OM starts in a single nail but soon other nails will be invaded and it may lead to TDO[18]

2)SUPERFICIAL WHITE ONYCHOMYCOSIS[SWO]

This type of OM is the less common form.It involves the dorsal surface of the nail and present as a well-defined powdery white patches and often away from the free edge. It usually involves the toe nails and present with a range of dyschromias depending on the organism involved; hence the term "superficial white onychomycosis" is restrictive.

Superficial black onychomycosis caused by *Trichophyton rubrum*[39] and *Scytalidium dimidiatum*[40] have been described. In HIV patients both toe and finger nails are affected and may co-exist with PSO[19]. The most common etiologic agent is

T.mentagrophytes. Several NDM such as *Aspergillus* and *Fusarium* species have also been implicated.[40]

3)PROXIMAL SUBUNGUAL ONYCHOMYCOSIS[PSO]

This is the uncommon form which involves the posterior nail fold. This form is particularly associated with AIDS patient[19].

4)ENDONYX ONYCHOMYCOSIS[EO]

In this type of OM invasion begins from the top surface and it penetrates deep into the nail plate. It is seen with endothrix scalp infection caused by ***T.soudanense.***

5)TOTAL DYSTROPHIC ONYCHOMYCOSIS[TDO]

This is the final common presentation of severe infection of all type of OM. The nail plate will be completely destroyed.

6)MIXED ONYCHOMYCOSIS[MO][38]

In the same nail different pattern of nail plate infection will be present. The most common form among them are PSO and SWO,DLSO and SWO. The etiological agents are *T.mentagarophytes, T.rubrum, Acremonium spp.,Aspergillus spp., and Fusarium spp.,.*

7)SECONDARY ONYCHOMYCOSIS[SO]:

The fungal agent will invade nail plate following other seconday pathological conditions like psoriasis.

8) CANDIDAL ONYCHOMYCOSIS[CO][34]

This type of OM may present in one of the following four ways.

a) Chronic paronychia with secondary nail dystrophy

b) Distal nail infection

c) Chronic mucocutaneous candidiasis

d) Secondary candidiasis

ORGANISMS MOST COMMONLY ASSOCIATED WITH DIFFERENT TYPES OF ONCYHOMYCOSIS : [5]

TYPE	ORGANISMS
DLSO	Dermatophytes (*T.rubrum,T.mentagrophytes,E. floccosum)* *Fusarium spp.,* *Scopulariopsis brevicaulis* *Scytalidium spp.,* *C.albicans*
SO (White or black)	*T.mentagrophytes, T. rubrum* *Fusarium spp., Acremonium spp.,* *Scytalidium spp.,*
EO	*T.soudanense* *T.violaceum*
PSO	*T.rubrum, Fusarium spp.,*
Mixed pattern DLSO plus SWO SWO plus DLSO SO plus PSO DLSO plus PSO	 *T. rubrum* *T. rubrum, Fusarium spp.,* *T. rubrum, Fusarium spp.,* *T.rubrum*

TYPE	ORGANISMS
TDO	Dermatophytes *Scytalidium spp.,* *C. albicans*
Paronychia With onychomycosis (usually DLSO or PSO) Without onychomycosis	*Candida* species *Fusarium spp.,* *Scytalidium spp.,* *Candida* species *Fusarium spp.,*

SEVERITY OF ONYCHOMYCOSIS[98]:

Based on the number of nails involved and extent of nail involvement OM can be garaded as mild,moderate and severe .In the mild degree, ≤25% involvement or ≤ 4 nails involved.In moderate degree 26 to 74% nail involvement or 5 to 8 nails involved and in severe form ≥ 75% nail involvement or ≥ 9 nails involved.

PATHOGENESIS:[28],[18]

DERMATOPHYTES

STAGE OF ADHERENCE

Dermatophytes produce certain keratinolytic substance.The proteinases produced by the fungal agent will hydrolyse the keratin and helps in the adherence and peneteration of arthroconidia. Also depends upon the chemical and mechanical factors.

STAGE OF PENETERATION:

The keratinase activity of dermatophytes can be induced by a low molecular weight protein peptides which are released from the epidermis by the action of other

fungal proteinases. So the fungal proteinase produced by other fungal agents may have a role.

HOST RESISTANCE AND IMMUNOLOGY

Dermatophytes are keratinophilic organisms. They quickly digest keratin.

The nail provides easy portal of entry to many pathogens , through the proximal nail fold and the distal nail fold. Although, nails are protected by the cuticle and the distal sole horn, respectively .

Nail is considered to be one of the relative immune privilege site due to a very low level of expression of MHC (Major histocompatibility) Class I antigens, local production of potent immunosuppressive agents, dysfunction of antigen presenting cells (APC) and inhibition of Natural Killer (NK) cell activity.

VARIOUS PROTECTIVE MECHANISMS

Nail units have a strong innate immunity.

Dorschner *et al* has shown the increased expression of local antimicrobial peptide (human cathelicidin LL-37) [29]. Normally cathelicidin LL-37 is not expressed in human skin, it will be induced on exposure to infection or inflammation.Although, it is strongly expressed in nail unit. It has a potent activity against *Pseudomonas aeruginosa*[30] and *Candida albicans*[29] because of its antimicrobial activity. A different pattern of distribution of immune cells was observed in different parts of the nail apparatus. In the proximal nail fold (PNF) the density of CD4+ cells is very high and very low density in the proximal nail matrix (PNM).[31]Around PNF, nail bed, and PNM the CD8+ T cells are rarely seen . The distribution of Langerhans cells are higher in epithelium of the nail bed and PNF when compared to that of the nail matrix. [33]

Thus due to a lack of effective cell-mediated immunity, the nail apparatus is susceptible to invasion by fungal organisms, if it gets exposed due to various predisposing factors. Hence, OM is actually a chronic infection without any inflammation.

Ventral and middle layers of the nail plate are invaded by the most dermatophyte species.

Highly irregular topography along with parallel ridges and grooves in the ventral surface provides the excellent site for the fungal hyphae to penetrate the nail plate[33]. The intermediate layer is less commonly involved.The dorsal nail plate is rarely involved . Hardest part is the dorsal nail plate.

The fungal pathogenicity also depends on the species, *Trichophyton mentagrophyte*s being a more active destroyer than *Trichophyton rubrum.* This active pathogenecity for the nail may be due to mechanical or enzymatic process.

The invasion of nail plate by dermatophytic species in *in vitro* models have been illustrated by Rashid *et al*., with *T. mentagrophytes*[33].

YEAST

Candidal OM were associated in the individuals with H/O repeated minor trauma or in those individuals who involve wet work. [32]

MOULDS :

Moulds are non keratolytic fungi.So, they will depend on the unkeratinized intercellular cement otherwise it will take the advantage of partial denaturation of nail keratin by pre-existing disease or trauma. Most often it cause infection following trauma or pre-existing disease. The moulds usually colonize the outer layer of the nail.

Infections are more common in toe nails. Moulds will produce a frond like mycelium. The fungus will grow inbetween the layers of keratin with the help of these fronds by digesting the intercellular substance as they grow [45]. *S. brevicaulis* and most *Aspergillus* species are capable of sporing *in vivo* and these spores can be seen on direct microscopy of nails.[86].

ETIOLOGICAL AGENT OF ONYCHOMYCOSIS:

Dermatophytes are considered to be the primary pathogens(90% in toe nails and 50% in finger nails) followed by yeast. Previously, non dermatophytic mould(NDM) which were merely considered as contaminants ,but nowadays they are the emerging pathogens[41] .

ETIOLOGICAL AGENTS OF ONYCHOMYCOSIS[5],[11]

DERMATOPHYTES	NON-DERMATOPHYTE MOULDS	YEAST
T. mentagrophytes	*Acremonium* species	*C. albicans*
T.rubrum	*Alternaria* species	*C.parapsilosis*
T. soudanense	*Aspergillus* species	*C. tropicalis*
T.violaceum	*Fusarium* species	*C.guilliermondii*
E. floccosum	*Onycochola canadensis*	*C. lusitaniae*
M. gypseum	*Geotrichum candidum*	
M. canis	*Cladosporium carrionii*	
	Scopulariopsis brevicaulis	
	Penicillium species	
	Scytalidium dimidiatum	
	Scytalidium hyalinum	
	Zygomycetes	

DERMATOPHYTES[50,51,88][SKIN PLANT]

Dermatophytes are the major pathogen. They are hyaline septate moulds. The hyphae of these organism will peneterate the stratum corneum of nails by means of proteases.Dermatophytes are classified into 3 genera ,comprising 39 closely related species

.

- *Trichophyton* species affects skin ,hair and nails.The *Microsporum* species affects skin and hair. The *Epidermophyton* species affects skin and nail.
- The telomorphic state has been described for only 23 dermatophytes.

Based on the ecology and host preferences they are catagerised into 3 groups

ANTHROPOPHILIC	ZOOPHILIC	GEOPHILIC
T. concentricum	*T. simii*	*T.ajelloi*
T.gourvilii	*T.mentagrophytes*	*T.terrestre*
T. megninii	*var.mentagrophytes*	*M.gypseum*
T. mentagrophytes var interdigitale	*T.verrucosum*	*M.praecox*
T.tonsurans	*T.equinum*	*M.cookei*
T. rubrum	*M.canis*	*M.fulvum*
T. schoenleinii	*M.equinum*	*E.stockdaleae*
T.soudanense	*M.persicolor*	
T.violaceum	*M.nanum*	
T.yaoundei	*M.gallinae*	
M.audouinii		
M.ferrugineum		
E.floccosum		

MODE OF TRANSMISSION

CATEGORY	MODE	CLINICAL FEATURES
ANTHROPOPHILIC	Human to human	Mild,noninflammatory;chronic
ZOOPHILIC	Animal to man	Intense inflammation;acute
GEOPHILIC	Soil to human or animal	Moderate inflammation

The most isolate among dermatophytes is *T.rubrum* of about 23 %-38% [52][53][28]. Other etiological agent most commonly involved in OM were *T. mentagrophytes,T.verrucosum,E.floccosum and Microsporum* species[54].

YEAST:

They are continually evolving and the medically important species belong to 2 classes.Saccharomycetes which contains *Candida* species and Tremellomycetes which contains Basidiomycetes fungi *Trichosporon* and *Cryptococcus*. *Malaseezia* species is also a basidiomycetes but it belongs to a different subphylum ,the Ustilaginomycetes[78].

CANDIDA:

They are gram positive oval budding yeast cell**.** There are 200 anamorphic species of genus *Candida* and placed within ascomycetes.The commonest pathogenic species is *Candida albicans*.The telomorphic state have also been demonstrated for various species of *Candida* which includes *Clavispora, Debaryomyces, Kluyveromyces, Pichia* and *Yarrowia*[78].

VIRULENCE FACTORS[44][96]

1) Adhesins,helps in binding of *Candida* species to the endothelial and epithelial cells.

2) Enzymes - aspartyl proteases,serine proteases,lipases,esterases,phosphatases. These enzymes helps in facilitating the hyphal invasion.

3) Complement receptors : *Candida albicans* have the ability to bind to the complement derived opsonins.
4) Phenotypic switching : A single strain can switch reversibly among different colony phenotypes & thereby they evade the host immune system.
5) Able to form bio-flim.

CANDIDA AND ONYCHOMYCOSIS:[34], [35],[1]

It is more common in females. *Candida* yeast infection of nail plate will present in one of the following four ways.

A) CHRONIC PARAONYCHIA WITH SECONDARY NAIL DYSTROPHY

The most common form of CO.This form generally occurs in patients with wet occupations.Secondary to chronic immersion in water the posterior nail fold will be inflamed.The cuticle will get detached from the nail plate and lose its water-tight properties.Then the yeasts will invade subcuticular space and leads to further swelling of posterior nail fold.

B) DISTAL NAIL INFECTION :

Uncommon with yeasts.These patients will have some form of vascular insufficiency.

***C) CANDIDA* GRANULOMA:**

It occurs in individiuals with CHRONIC MUCOCUTANEOUS CANDIDIASIS. This form occurs in less than 1% of onychomycosis. The entire thickness of the nail plate will be affected . In advanced cases, pseudo-clubbing will occur.

D) SECONDARY CANDIDIASIS:

Follows other disease of nail,mostly psoriasis. *Candida* has been documented as the most common pathogen in OM around 46% [43]. *C.albicans* are particularly associated with finger nails in females. *C.paropsilosis* is the most common pathogen among *Candida* species followed by *C.albicans,C.gulliermondii, C.tropicalis& C.lusitaniae*[42].

MALASEEZIA SPECIES IN ONYCHOMYCOSIS

The causative role of *M. furfur* in OM is a diagnostic problem because its keratinolytic ability has not been demonstrated[79]. Presence of *Malassezia* in the subungual debris is very important, because in intensive care unit patients it serves as a source of systemic infections and is contagious[80][81].

NON-DERMATOPHYTE ONYCHOMYCOSIS:[NDO]:

Moulds were considered traditionally as uncommon secondary pathogens of diseased nails. However it constitutes about 15% of total case of OM. NDO are suspected when the patient has prominent periungual inflammation.The prevalence of non-dermatophytic moulds(NDM) varies with the geographical distribution of the population and also depends on the diagnostic criteria used.It is common in males and among immunosuppressed individuals.The most common type of presentation is DLSO

followed by SWO[46].The most common moulds are *Scopulariopsis brevicaulis, Aspergillus* species, *Scytalidium dimidiatum, Scytalidium hyalinum, Hendersonula toruloidea, Fusarium* species, *Chaetomium globosum* [47][85].

Different studies globally have reported various rate of prevalence of NDM ranging from 2.3% - 45.8%[49].

A 10 year study conducted in Korea revealed that 2.3% of OM was associated with NDM. 84.7% had toe nail onychomycosis and 15.3% had finger nail involvement . 13.6% of them had DM[48].

OTHER RARELY ISOLATED MOULDS[86]

SPECIES	**REFERENCES**
Aspergillus sclerotiorum	Feuilharde de Chauvin and de Bievre 1985;
Aspergillus ustus	Garcia- Martos *et al* 2001
Aspergillus unguis	Wishe and English 1996
Curvularia lunata	Schonborn and Schmoranzer1970
Exophila jeanselmi	Barde and Singh 1983 Velez and Diaz 1985
Geotrichum candidum	Boisseau –Garsaud *et al* 2002
Penicillium species	Restrepo *et al* 1976,Velez and Diaz 1985
	Walshe and English 1996, Velez and Diaz 1985

To increase the predictive power of diagnosis of nondermatophytic invasion of a nail, according to Summerbell (35) the nondermatophytes isolated in nail tissue should be categorized as one of the following:

1. Contaminant species .
2. Normal mammalian surface commensal organism.
3. Transient saprobic colonizer . (colonizer of accessible surface molecules but non-invasive)
4. Persistent secondary colonizer - colonizer of material infected by a dermatophyte but incapable of remaining after the dermatophyte is eliminated.
5. Successional invader – The species which can cause infection after gaining entry into a nail via the disruption caused by a primary pathogen .
6. Primary invader which are able to cause OM in a previously uncolonized nail.

Diagnosis of NDM is difficult and it requires more than one laboratory analysis for confirmation.

The criteria of English *et al.*, [36] stated that

1. If a dermatophyte is isolated - Considered as the pathogen.
2. To consider the mould and yeast as pathogen:
    - KOH mount should be positive(arthrospores,hyphae,yeast cells)
    - There should be at least five colonies of the same mould (out of 20 nail fragments plated per person) with the absence of dermatophytes.

However it is difficult to fulfill the criteria, and it leads to high false negative results.

Therefore, **Shemer *et al.*,** suggested that when NDM is isolated in the first culture, then the patient should be re-examined and three separate nail samples should be taken. If NDM is confirmed in all of the three cultures taken, then it is considered to be significant and we have to treat accordingly.

DIFFERENTIAL DIAGNOSIS[47]:

It is difficult to diagnose subungual tinae unguium because of scarcity of fungi and the fungus is located in the lower most part of the nail.

These are the following conditions which mimic Onychomycosis

- **CONGENITIAL CONDITIONS**

  Non mycotic leukonychia,clubbing,Beau lines,Pachyonychia congenital

- **EXTERNAL CONDITIONS**

  Onychogryposis,Onychophagy,ingrowing toe nails

- DERMATOLOGICAL DISEASE CONDITIONS

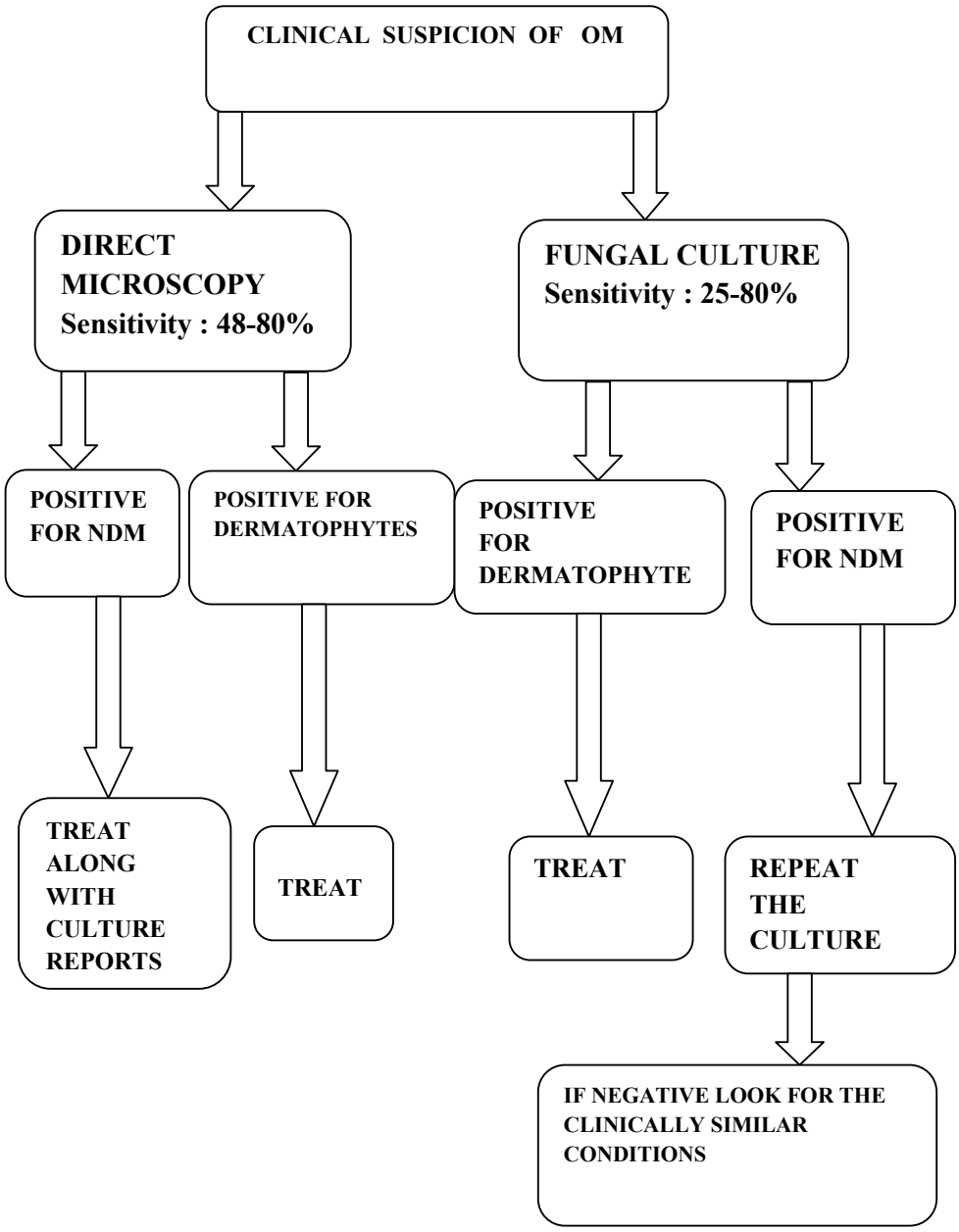
  Eczema,Lichen planus,Bacterial paronychia,Dariers disease,

  Raynauds disease,Psoriasis,Nail bed tumors.

Among these, psoriatic nail is exteremly difficult to diagnose. The systemic disease will affect several nails and symmetric in distribution.But in T.unguium it will involve only single nail and it is assymmetric in distribution.

LABORATORY DIAGNOSIS:

ALGORITHM FOR DIAGNOSIS OF ONYCHOMYCOSIS[28]



SAMPLE COLLECTION

Collection of appropriate sample is very important for successful laboratory diagnosis. Sample collection method differs with the type of OM[11].

For all types of OM,nails should be first cleaned with alcohol to eliminate the contaminants like bacteria[35].

DLSO

Samples should be collected from the nail bed because the concentration of viable fungi is the greatest[68]. Using nail clippers the nails should be clipped short. Using a small curette or no.15 scalpel blade the samples should be collected from the nail bed as close to the cuticle[35].

PSO

Using a sharp curette scrapings should be taken from the infected nail as proximal as possible to lunula[55].

SWO

Using no.15 scalpel blade, first few scrapings from the outermost surface should be discarded and then the underlying debris is collected.[67]

EO

Scrapings are taken from the discolored portions[55].

CANDIDAL OM

Scrapings are taken from the proximal and lateral nail fold[55].

TDO

Scrapings can be taken from any of the diseased part of nail plate or bed[11].

The samples collected by means of drilling technique has been found to have improved culture sensitivity.Vertical drilling technique has been found to useful in PSO.To obtain full thickness of the nail, nail clippers should be used[28] .

Strong black paper or black photographic paper can be used for sample collection because it will gives better visualization regarding the amount of scrapings to be obtained[62].The paper also allows the specimen to dry out so that it helps in reducing bacterial contamination[75].Then the paper can be folded tightly and the corners are sealed.

Two commercial package systems for transport is available.

1) Myco Trans(Bigger,UK)

2) Derma pak(Dermaco,UK)

CONVENTIONAL METHODS

DIRECT MICROSCOPIC EXAMINATION

KOH MOUNT[62]- A simple,rapid and inexpensive method to confirm the diagnosis. The sample is incubated in 10-30% KOH(potassium hydroxide) solution .KOH will digest keratin, so that the fungal hyphae will be clearly visible. The nail specimens can take a longer time to clear. The subungual debris can also be examined within 10 minutes with 10-15% KOH. If the nail sample is larger it should be made up into smaller pieces and then incubated at 37ºC for one minute and then examined[28].40% DMSO can also added along with KOH because DMSO will facilitate clearing .Alternatively NaOH with 5% glycerine can also be used[62] .The

sensitivity rate of KOH varies from 48% to 80%[28]. To improve the visualisation Parker's Blue black ink can also be used along with KOH[11].

Calcofluor white stain and other special stains can also be used for direct examination. Recently, Lim and Lim reported the use of Chicago sky blue(CSB) stain to improve the sensitivity as well as specificity . Under 40X magnification fungal elements can be easily visualized on normal bright-field microscopy. However,species differentiation cannot be done by direct microscopy [28].

HISTOPATHOLOGY

The current Gold standard method for the diagnosis of OM is PAS (periodic acid Schiff stain)[59]. For histopathological examination, nail clippings are taken and fixed with formalin for 4-8 hours and then softened [using agents like Potassium hydroxide (KOH), 5% trichloroacetic acid, 10% Tween- 40, or chitin softening solution containing mercuric chloride]. Then the softened sample is fixed in 10% buffered phosphate formalin for 24 hours, dehydrated and then embedded. Using a microtome,semi-thin sections (5 microns) are taken and then stained with periodic acid-Schiff (PAS). This procedure takes for about 24-48 hours.

Sufficient amount of subungual debris is enough to confirm the diagnosis and even the nail plate may not be required[60].

PAS is found to be the most sensitive test with a sensitivity rate of 82%. The sensitivity of culture is 53% and direct microscopy is 48%.[66]Histopathology is valuable because it gives a clue of level of invasion and arrangement.Staining with Grocott methenamine-silver (GMS) was shown to be superior to PAS because it gives

a better contrast between fungus and surrounding materials. But, the procedure is more complex[28].

HPE is useful for differentiating the dermatophytes, yeast and NDM and allows the examination of mycelia threads and spore morphology in detail. However, it is a time consuming procedure which needs to be overcome.

CULTURE[28]

Isolation of the fungal pathogen from nail is more difficult.The sample taken from the distal end of nail are usually non-viable,so it will not grow in culture. The hyphae in the proximal end of the nail are viable.The culture results can be improved if samples are taken from more proximal sites. Because of discomfort, it is difficult to obtain proximal sample.

Previously, the culture was considered as the gold standard techinque of diagnosis, which can identify the involved fungus. The sensitivity of culture varies from 25 to 80%[56]. False negative results accounts for about 30% of cases, particularly when the sample is insufficient, taken from distal portions or is not crushed prior to inoculation[56],[57].

Specimens should be inoculated onto two different media. Sabouraud dextrose agar with gentamicin to isolate all the fungi including yeasts and NDM and a Sabouraud dextrose agar with cycloheximide and chloramphenicol for isolation of dermatophytes . Cylohexmide will inhibit the growth of NDM.Cultures are incubated for three to four weeks and examined weekly. The isolates are identified on the basis of the growth pattern,macroscopic appearance and microscopic examination. If both the

media shows growth ,the etiological agent is dermatophyte. If the growth is seen only in cycloheximide free medium,then the etiological agent may be NDM[28].

DERMATOPHYTE TEST MEDIUM(DTM)[58]

This is an alternative culture media originally described by Taplin *et al* for presumptive identification of dermatophytes. The growth is indicted by the change in colour of the medium as a result of pH change.(YELLOW TO RED).The medium contains cyclohexamide which will inhibit the growth of saprophytic fungi and gentamicin which inhibits the growth of bacteria. The cultures can be identified within one week.But it may give rise to false positive results.Dermatophyte test medium can be used as alternative for presumptive identification of dermatophytes.

DERMATOPHYTE IDENTIFICATION MEDIUM:[61]

It is also used as presumptive identification medium of dermatophytes.Here the medium changes from greenish blue to purple within 24 to 48 hours. The false positive results are less than that of DTM.

OTHER SPECIAL MEDIA:

BROMOCRESOL PURPLE MILK SOLID GLUCOSE MEDIUM[62]:

It is a differential medium used for identification of dermatophytes.Growth is indicated by colour change in the medium.Available commercially as Dermatophyte milk agar. Type of growth and change in pH are useful in differentiating *T.rubrum* from T.*mentagrophytes,T.mentagrophytes* from *M.persicolor.*

CORN MEAL AGAR WITH 1% DEXTROSE[62]

This medium is used to differentiate *T.mentagrophytes* from *T.rubrum*.Here the dextrose will promote the growth and pigment production of *T.rubrum*.

TRICHOPHYTON AGAR 1 TO 7 [62]

Based on the growth requirements the *Trichophyton* species can be identified.They are a set of 7 media with different growth factors.Growth in all the seven media is scored and the species are identified.

SPECIAL MEDIA FOR SPORULATION INDUCTION[62]:

The sporulation can be induced in nutritionally deficient medium such as Potato Dextrose Agar,Potato Flake Agar,Corn meal agar,Corn meal glucose agar.

INVITRO HAIR PERFORATION TEST[62]:

This test distinguishes between the atypical isolates of *T.mentagrophytes* and *T.rubrum*.It is also used to distinguish *M.canis* and *M.audouinii* and also between *M.praecox* and *M.gypseum*.

POSITIVE (Wedge shaped perforation seen perpendicular to the hair shaft)	**NEGATIVE (No perforation)**
T.mentagrophytes	*T.rubrum*
M.canis	*M.audouinii*
M.gypseum	*M.praecox*

UREASE TEST[[62]]

The ability of the pathogen to hydrolyse urea can be utilized to differentiate *T.rubrum*(Negative) from *T.mentagrophyes*(Positive).

NEWER METHODS

POLYMERASE CHAIN REACTION[28]

Nowadays various molecular techniques using PCR assay have been evaluated. It is an accurate, rapid and stable method for identifying pathogenic fungi from both the nail samples and from the fungal colonies. The methods used in samples from cultured colonies include Arbitrarily primed PCR, real-time PCR, PCR-restriction fragment length polymorphism (RFLP), PCR-direct sequencing and double-round PCR are the methods used for identifying the pathogen in cultured colonies.Specificity of PCR is excellent. Disadvantage of this method is risk of contamination and it cannot differentiate pathogenic from non pathogenic fungi.[63]

To detect the etiological agent from the nail sample directly Li *et al*., developed and evaluated the efficacy of triplex PCR procedure which had a sensitivity and specificity of 93.3% and 100% respectively.This method will distinguish the three groups of fungi in OM and could be completed within 8 hours. [63]

OPTICAL COHERENCE TOMOGRAPHY

This method is non-invasive.It employs non-contact cross-sectional imaging of biological tissue and detects the back scattering near infrared light of the inhomogenities within the sample.The hyperkeratotic nail will have lower light scaterring area.The high signal intensity is seen with hypal element due to their chitin concenteration[65] . This method is superior to KOH and cultures.Hence, it is a reliable,

easy, noninvasive method to visualize fungal elements *in vivo*.[65] Its availability and cost effectiveness are not favorable for use in clinical practice.

CONFOCAL LASER SCAN MICROSCOPY[28]

An *in vivo* test used in diagnosis of OM[64]. It is unsuitable for routine use, because it is expensive and complicated . Several reports shown that misdiagnosis is common and it is a time consuming procedure.

MATRIX-ASSISTED LASER DESORPTION/ IONIZATION

TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS)

This method is based on detection of biochemical characteristics as a result of the activity of noninfectious diseases or due to mycological infections. This technique analyses the protein patterns of nail samples. Identification is by comparing the known peptide pattern from various nail disoders which is already existing in data bases with that of the peptide pattern in nail sample. A distinct advantage of this method over the conventional method(KOH and Culture) is its ability to differentiate between OM and non-fungal nail disorders This method is fast and results are available within 24hours.Sample preparation is also simple[28].

PHASE CONTRAST HARD X-RAY MICROSCOPY

This method uses phase contrast microscopes utilizing synchrotron radiation. The internal structures of the sample can be imaged precisely because of high resolution and brightness.This method does not require fixatives or stains[28].

IDENTIFICATION OF *CANDIDA* SPECIES

GERM TUBE TEST[REYNOLD'S-BRAUDE PHENOMENON]

Germ tube test is used to differentiate between the *Candida albicans* and non-albicans species.

SUGAR FERMENTATION TEST

This test can be used to differentiate between the *Candida* species based on the fermentative patterns.

SUGAR ASSIMILATION TEST

This test determines the ability of the yeast to utilize the particular carbon compound as sole source of energy under aerobic conditions. These tests can be performed on liquid or solid medium.

a) LIQUID MEDIA

Wickerham and Burton (1948) and Wickerham (1951) described the test by employing liquid media.

b) SOLID MEDIA : can be done in two ways.

1) Auxanographic method of Beijerinck (1889) By suspending the yeast in agar in pour plates and the individual test sugars are placed at intervals around the circumference.

2) In another method ,the test compound is incorporated onto a nutrient agar basal medium and the test isolate is inoculated as a streak on the surface of the petri plates.

The **disadvantage** of agar plate is it tends to dry within a few days ,so delayed growth reactions cannot be detected.

But the **advantage** of using petri plates are

- The results can be read within 2 to 4 days
- Any contaminating organisms if present can be easily seen

Auxanographic method is more reproducible than the Wickerham method because of its simplicity and ease in interpreting results[83].

CORN MEAL AGAR MEDIUM

Used to study the morphology of yeast such as blastoconidia, chlamydoconidia,true hyphae,pseudohyphae and arthroconidia.

CHROMagar Candida medium

It is a differential and selective chromogenic medium to identify *Candida* species. These media are based on direct detection of specific enzymatic activities by adding substrates of flurochromes to media.CHROM agar Candida medium is useful in rapid and presumptive identification of *Candida* species within 48 hours[82].

NITRATE ASSIMILATION TEST:

It tests the ability of the yeast to utilize nitrate as a sole source of nitrogen source.However,it has limited value for identification of yeast and are primarily used for discrimination of *Cryptococcus* species.

RAPID TREHALOSE TEST

It helps in the presumptive identification of *C.glabrata* within 3 hours[78].

COMMERCIALLY AVAILABLE YEAST IDENTIFICATION TESTS[76].

- API-20C AUX system is based on carbohydrate substrate utilizaton profiles.The limitation of the system is that it cannot identify the unusual species.

- Microscan Yeast Identification Panel

  This system uses the chromogenic substrates to asses the enzymatic activity.

- VITEK Biochemical Cards-The yeast biochemical card is a 30 well, disposable plastic card that contains conventional biochemical tests and negative controls.It is used with the automated Vitek II system used for bacterial identification .The overall accuracy of the system in identification is nearly 100% when compared with API-20C AUX system.

- Matrix-Assisted Laser Desorption Ionization Time-Of-Flight(MALDI-TOF)

  This method is emerging as a rapid identification technique of yeast and fungal isolates,will reduce the turn around time.

MOLECULAR METHODS:

PCR-RFLP METHOD[93]:

Rasoul *et al* used PCR-RFLP method in speciating 322 *Candida* isolates.ITS1-ITS2 region of the extracted yeast isolate is amplified by PCR method,PCR products are digested by MspI restriction enzyme.Within the *C. parapsilosis* group, SADH gene was furher amplified and the products were digested by NlaIII restriction enzyme to discriminate *C. parapsilosis, C. metapsilosis* and *C. orthopsilosis*.

TREATMENT OF ONYCHOMYCOSIS[89]:

It is very important to treat OM because it does not resolve spontaneously and if left untreated it can spread to other nails and serves as a reservoir of infection for other peoples.OM is very difficult to treat because of long growth period of the nail,hardness of nail plate and the location of infection between the nail bed and nail plate. The therapy also depends on many factors such as the site of involvement, severity of the disease, the etiological agent, number of nails, concomitant drugs and choice of drug by the patient/physician[87].

The treatment of OM in diabetics is same as that of non-diabetic individuals. The elderly DM individuals require longer duration of therapy because nails grow more slowly among them.The DM patients with onychomycosis have a higher rate of secondary infections and complications .The rate of relapse is higher among the elderly patients above 55 years of age[89].

The several treatment modalities used in onychomycosis among diabetic patients are topical, systemic, combination therapy and nail removal.

1) TOPICAL AGENTS:

The three classes of topical antifungal agents available are imidazoles (e.g., clotrimazole), polyenes (e.g., nystatin), and allylamines- benzylamines (e.g., terbinafine). All of the three agents are effective against *Candida*, but only allylamines-benzylamines and imidazoles are effective against dermatophytes. Because of inadequate penetration of the drugs into the affected site of tissues and nail bed,topical agents are not effective in clearing the nail infections.The topical agent is useful in SWO, because the pathogen grows on the upper surface of the nail plate.

Antifungal nail lacquers such as amorolfine and ciclopirox are available in treating OM and penetrates the nail better than the creams and gels.

Topical antifungal agents are useful in the reduction of reinfection and relapses once the initial infection have been treated completely .

2) SYSTEMIC

The standard oral therapy for OM was griseofulvin for more than 30 years. It is active against dermatophytes only,also has several drug interactions and and adverse effects.The cure rate is less than 40%.Because of these reasons, it is rarely used nowadays.

The imidazole group of antifungal agents are active against *Candida*,dermatophytes and non-dermatophytes.But they also has significant side effects and drug interactions.

A triazole antifungal agent ,Itraconazole has fewer side effects.It is effective against *Candida* ,dermatophytes and some moulds. Azole antifungals, fluconazole and itraconazole , have been shown to elevate the levels of oral hypoglycemic agents. Itraconazole has been found to be effective and safe in DM patients at a dose of 200 mg twice daily. No statistically significant changes in hemoglobin A_{1c} levels have been noted in diabetic patients receiving pulse itraconazole for 3 months. The mycological cure rate of OM is 79% after 6 months of therapy. Pulse therapy has been shown to be just as effective as the continuous therapy.

Candidal OM can be treated effectively with fluconazole.

Terbinafine, an allylamine antifungal agent, is the drug of choice in treating onychomycosis. It is highly active in *in vivo* against dermatophytes , however not active against *Candida* and mould species.There is no significant interactions with oral hypoglycemic drugs.Terbinafine is relatively safe in DM patients and it is acceptable for long-term maintenance of the healthy nails in diabetic patients.

Terbinafine, 250 mg once daily for 3 months, has been shown to achieve a mycological cure rate of 71% in fingernail OM and 82% in toenail OM.Pulse therapy with terbenafine has been formulated and it is not efficacious as continuous therapy.

3) COMBINATION THERAPY

The newly developed treatment option is combination of oral and topical antifungal drugs, which will increases the likelihood of cure. One study repoted that the efficacy of terbinafine has been improved when combined with topical amorolfine[90]. Another study showed the improved efficacy of continuous itraconazole when combined with topical amorolfine[91]. The mycological cure rate of continuous terbinafine is 88.2%,when it is combined with topical ciclopirox mycological cure rate is 64.7%.

4) SURGICAL

It is used as an adjuvant therapy,involves the removal of nail either chemically or surgically. Surgical removal of nail is rarely used in treatment of OM in DM patients because of their increased risk of secondary infectons,poor wound healing and gangrene.

Urea ointment is used in chemical avulsion .It is preferred to surgery because it is a painless method[87].

Depending on the age and health of patient, side effects, drug interactions, dosage schedule, cost of treatment and patients compliance the physician should choose the treatment option[87].

CLINICAL CURE:

The term clinical cure means disappearance of all lesions on each nail or residual disease of no more than 10% of original disease surface[88].

MYCOLOGICAL CURE:

Negative culture and negative microscopy indicates mycological cure[88] .

EDUCATION:

Education of the patient plays an important role in treating the infection.High-risk diabetic patients, those with peripheral vascular disease or peripheral neuropathy, should be educated properly about foot and leg examinations.

ANTIFUNGAL SUSCEPTIBILITY TESTING :

Relapses are common in OM patients. By doing *in-vitro* antifungal susceptibility an effective antifungal agent can be selected for treatment and thus helps in optimizing the theraphy[69].The resistance may be intrinsic or acquired[84].

FACTORS THAT ARE RESPONSIBLE FOR CLINICAL ANTIFUNGAL RESISTANCE[84]

HOST FACTORS	FUNGAL FACTORS	DRUG FACTORS
Immune status Severity of infection Site of infection Poor adherence to regimen Presence of foreign material	CELL TYPE Morphology Serotype Bioflims Cell states Genomic stability of the strain Species and strain Size of population Strain MIC Population bottle necks	PHARMACOKINETICS Absorption Distribution Metabolism DOSING Freqency Quantity Cumulative dose Fungistatic nature of drug Drug- drug interactions

The antifungal susceptibility testing are based on the standards set by The Clinical Laboratory Standard Institute(CLSI).

Document M38-A2 describes the standard reference method for Antifungal susceptibility testing of filamentous fungi by broth dilution method. The standards for susceptibility tesing are very specific in the test medium,inoculum size,incubation temperature and time,pH and end point determination.The medium recommended is RPMI 1640 (with glutamine,without bicarbonate and with phenol red as indicator,pH=7.0).A 7 day old culture on PDA should be used for inoculum preparation. DMSO should be used to dissolve water insoluble drugs.The inoculated microtiter plates should be incubated at 25°C for 48 hours.

M27-A3,Reference method for Antifungal susceptibility testing of yeasts by broth microdilution method.A 24 hour old culture should be used and turbidity should

be matched with 0.5Mc Farland opacity standard. The inoculated microtiter plates should be incubated at 37°C for 24-48 hours.

M44-A2,describes the method antifungal susceptibility testing of yeast by disk diffusion method[74].

A good correlation between the *in vitro* data(broth microdilution method) and clinical outcome has been documented[73].

However a standard reference method for dermatophytes is not available[69]. The most important aspects of doing antifungal susceptibility test are the inoculum size and its constitution, the incubation time and temperature which have an influence on the susceptibility result[70][71][72].

AIMS & OBJECTIVES

AIMS & OBJECTIVES

- To identify the etiological fungal pathogens in onychomycosis among diabetic patients.

- To determine the epidemiological pattern of the pathogens associated with onychomycosis.

- To study the antifungal susceptibility pattern of the isolates.

MATERIALS & METHODS

MATERIALS AND METHODS

PLACE OF STUDY:

This study was conducted at The Institute of Microbiology in association with Institute of Diabetology and Department of Dermatology , Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai .

STUDY DESIGN:

Cross-Sectional study

STUDY PERIOD

The study period was for one year from October 2013 to September 2014.

ETHICAL CONSIDERATION

Approval was obtained from the institutional ethics committee before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were recruited for the study.

INCLUSION CRITERIA:

All known diabetic patients above the age of 18 years who were clinically diagnosed as onychomycosis, irrespective of their current glycemic status were included.

EXCLUSION CRITERIA:[97]

- Patients on topical antifungal agents for past four months .
- Patients on systemic antifungal agents for past nine months.

DATA COLLECTION:

Patients' name,age,sex ,address, occupation, duration of OM , immune status of the patient, H/o trauma, type of DM, duration of DM, blood glucose levels were noted.

CLINICAL EXAMINATION:

The patient was examined for the following:

Type of OM, colour of the nails, number of nails involved, toe/finger nails, associated conditions like psoriasis, concomitant dermatophytic fungal infections affecting skin/hair.

STUDY POPULATION

Sample size: 175

SAMPLE COLLECTION, TRANSPORT AND PROCESSING:

SAMPLE:

The nail scrapings and subungual debris were collected from all patients.

COLLECTION :

The surface of the nail was first disinfected with 70% alcohol to remove the bacterial contaminants.Then the nail samples were obtained according to the type of onychomycosis. A no.15 sterile scalpel blade was used to collect the samples.

DISTAL LATERAL SUBUNGUAL ONYCHOMYCOSIS

The nails were clipped short using nail clippers. The scrapings were obtained from the nail bed proximally to the site of infection.

SUPERFICIAL WHITE ONYCHOMYCOSIS

The first few scrapings were discarded and the underneath debris were collected.

PROXIMAL SUBUNGUAL ONYCHOMYCOSIS

The scrapings were obtained as proximal as possible to lunula of the infected nail.

CANDIDAL ONYCHOMYCOSIS

The scrapings were obtained from lateral and proximal nail fold.

ENDONYX ONYCHOMYCOSIS

The scrapings were taken from the discolored portions.

TOTAL DYSTROPHIC ONYCHOMYCOSIS

The scrapings were obtained from the proximal diseased part of the nail.

The scrapings were collected on a sterile glass slide and subjected to KOH examination and culture immediately.

PROCESSING

KOH MOUNT

20% KOH and 40% KOH were used for finger nails and toe nails respectively. The scrapings were placed on a sterile glass slide,a drop of KOH added and a coverslip placed over it. Then the slide was gently passed over the flame and examined under 10X and 40X objective of the light microscope. Then the slide was examined for the presence of hyphae, arthrospores, pseudohyphae and budding yeast cells.

TUBE KOH

Tube test for tube KOH was used for those nail samples which took longer time to dissolve. The nail sample was dissolved in 10% KOH and incubated at 37ºC overnight and examined under the light microscope next day.

CULTURE

The samples were inoculated immediately onto the following culture media.

1. Two tubes of Sabouraud Dextrose Agar with cyclohexamide(500mg/L) and chloramphenicol(50mg/L) were incubated at 22ºC and 37ºC for 4 weeks.
2. Two tubes of Sabouraud Dextrose Agar without cyclohexamide and chloramphenicol and with gentamicin(20mg/L) were incubated at 22ºC and 37ºC for 4 weeks.
3. Dermatophyte test medium was incubated at 22ºC for 2 weeks.

The non dermatophytic moulds and yeasts were considered as pathogen based on English and Walsh criteria[36]:

- A positive KOH mount
- More than 5 colonies in the medium and
- If the isolate yielded was the same in all three succesive cultures.

If the conidiation was poorly formed in SDA, the media was enriched with thiamine.

MACROSCOPIC EXAMINATION OF CULTURE TUBES

The culture tubes were examined regularly for fungal growth for upto 4 weeks in SDA and in DTM for 2 weeks.The duration of growth, colour and texture of the growth were noted and then subjected to microscopic examination.

MICROSCOPIC EXAMINATION

LPCB MOUNT

A drop of LPCB was placed a clean glass slide. A small portion of the colony was taken using a spade and placed on the mounting fluid. The mycelial fragments were teased apart with the help of two teasing needles. A coverslip was placed over it and examined under 10x and 40x objective of the light microscope. The type of hyphae,conidia and their attachment with condiophore/sporangiophore are examined and identified .Slide culture was performed for those isolates which could not be identified by LPCB mount.

SLIDE CULTURE:

The nutritionally deficient medium, PDA was used for the slide culture. A 1cm square agar block was cut out from PDA using a sterile scalpel. The agar block was transferred carefully with sterile precautions onto the sterile glass slide placed in a sterile petridish. A very small amount of the colony was inoculated onto the four sides of the agar block. One ml of sterile distilled water was added to a sterile container placed in the petri dish. The inoculated medium was incubated at room temperature and observed for growth. When good growth was observed, two permanent mounts were prepared. The coverslip was removed from the agar block with the help of sterile forceps and observed microscopically after adding LPCB. The agar block was also

removed and a drop of LPCB placed over the slide ,coverslip was placed and observed under 10x and 40x objective of the light microscope.

IDENTIFICATION OF DERMATOPHYTES:

Dermatophytes were identified and speciated based on macroscopic and microscopic features of culture. The other additional tests used to differentiate the various dermatophytes were as follows.

1) UREASE TEST:

Christensen's urea agar slope was inoculated and incubated at 25ºC for upto 7days.The hydrolysis of urea was indicated by a colour change to pink.This test was done to differentiate *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

2) IN-VITRO HAIR PERFORATION TEST:

A piece of sterile filter paper was placed in a sterile petridish containing 2ml of 10% diluted sterile yeast extract. Ten strands of sterile prepubertal hair of approximately 1cm in length was transferred onto the petridish using a sterile forceps, onto which the colony was inoculated.

The hair was incubated at room temperature for upto 4 weeks. A single strand of hair was placed on a glass slide,mounted with LPCB, coverslip placed and examined under microscope.

INTERPRETATION:

A wedge shaped perforation was considered as positive and suggestive of *Trichophyton mentagrophytes.*

Absence of wedge shape perforation was interpretated as negative and suggestive of *Trichophyton rubrum.*

IDENTIFICATION OF *CANDIDA* SPECIES:

Yeast and yeast like fungi were identified and speciated based on

1)GRAM STAIN

Presence of gram positive budding yeast cells, hyphae and pseudo hyphae was noted.

2) UREASE TEST

Christensen's urea agar slope was used.Urea hydrolysis was interpretated as positive when a change of colur to pink along with growth in medium were observed.This test was done to differentiate between the *Candida* species and other yeasts like *Malassezia* Species,*Trichosporon* Species ,*Geotrichum* Species *and Blastoschizomyces*.

POSITIVE	NEGATIVE
Malassezia species	*Candida* species*(except C.krusei)*
Trichosporon species	*Blastoschizomyces*
Candida krusei	*Geotrichum*

3) GERM TUBE TEST(REYNOLDS –BRAUDE PHENOMENON)

This test was done to differentiate *Candida albicans* from Non-albicans *Candida*. 0.5ml of human serum was taken in a sterile test tube, into which the yeast colonies were suspended. The tube was incubated at 37ºC for 2 hours in the incubator. After 2 hours a loopful of suspension was placed on a clean glass slide, a coverslip placed over it and examined under 40X objective of the light microscope.

INTERPRETATION:

Observation of germ tubes which are seen as a long tube–like projections with parallel walls and no constriction at the point of attachment to the yeast cell were interpreted as positive and was presumptively identified as *Candida albicans/Candida dubliniensis*.

4) CORN MEAL AGAR(DALMAU PLATE CULTURE)

This test was done to differentiate various species of *Candida*.

A corn meal agar plate containing 1% Tween 80 was divided into four quadrants. The suspected yeast colony was streaked by three parallel lines with a sterile straight wire onto it.A coverslip was placed over it, such that half of the streak extend beyond the coveslip.Then the plates were incubated at 25ºC for 2 days.

INTERPRETATION:

The petridish was placed on the microscopic stage after removing the lid and the edge of the cover slip was examined under 10X and 40X objectives.

The *Candida* species were identified based on the presence and arrangement of hypahe, pseudohyphae, chlamydocondia, blastoconidia and arthrocondia as follows.

ORGANISM	BLASTOCONIDIA	HYPHAE / PSEUDOHYPHAE
C.albicans	Spherical clusters at regular intervals on pseudohyphae	Chlamydocondia present on hyphae
C.tropicalis	Produced randomly along hyphae	Pseudohyphae present
C.kefyr	Elongated lie parallel to pseudohyphae	Pseudohyphae present
C.parapsilosis	Present but not characteristic	Large giant hyphae present
C.glabrata	Spherical small and tightly compacted	None produced

5) CARBOHYDRATE FERMENTATION TEST

This test was done using the peptone water fermentation test media with 2% sugars- Glucose,Lactose,Sucrose and Maltose. A pure colony suspension was inoculated into the media and incubated at 30 ºC for 3 days to 1week. The tubes were examined for acid production by change in colour to yellow and gas production in the durhams tube. The *Candida* species were identified based on the following fermentative pattern.

S.No	*Candida* Species	Glucose	Maltose	Sucrose	Lactose
1	*C.albicans*	F	F	NF	NF
2	*C.parapsilosis*	F	NF	NF	NF
3	*C.tropicalis*	F	F	F	NF
4	*C.pseudotropicalis*	F	F	F	NF
5	*C.krusei*	F	NF	NF	NF
6	*C.guilliermondii*	F	NF	F	NF
7	*C.glabrata*	F	NF	NF	NF

F-Fermented; NF-Not fermented

6) CARBOHYDRATE ASSIMILATION TEST(Auxanographic method)

Yeast nitrogen base agar media was prepared.A heavy yeast suspension was prepared from a 24 hour old culture in YNB and added to 18ml of the media , mixed,poured in the sterile petridish. The carbohydrate disks were applied on to the surface of the agar plate and incubated at 37ºC for 3 days.

The various carbohydrate disks used in this study were Glucose, Maltose, Sucrose, Lactose, Cellobiose and Trehalose.

INTERPRETATION:

Presence of growth around the carbohydrate disc was considered as positive for carbohydrate assimilation.

The *Candida spp* was speciated as follows:

S.No	*Candida* species	Glu	Mal	Suc	Lac	Cel	Tre
1	*C.albicans*	P	P	P	N	V	P
2	*C.parapsilosis*	P	P	P	N	V	P
3	*C.tropicalis*	P	P	P	N	P	P
4	*C.pseudotropicalis*	P	N	P	P	V	N
5	*C.krusei*	P	N	N	N	N	N
6	*C.guilliermondii*	P	P	P	N	P	P
7	*C.glabrata*	P	N	N	N	N	P

P- Positive, N-Negative, V- Variable. Glu –Glucose, Mal- Maltose, Suc- Sucrose, Lac-Lactose, Cel-Cellobiose, Tre-Trehalose.

7) CHROMagar CANDIDA MEDIUM

The yeast colonies were inoculated onto the medium and incubated at 37ºC for 48 hours.The colour of the colonies were observed and the organism was presumptively identified as follows,

SPECIES	COLOUR
C.albicans	Light-green
C.dubliniensis	Dark-green
C.glabrata	Pink to purple
C.krusei	Pink
C.parapsilosis	Cream to pale pink
C.tropicalis	Blue with pink halo

The *Candida* isolates were speciated based on the results of the germ term tube test,urease test along with phenotypic characteristics on corn meal agar, carbohydrate fermentation and assimilation test.

IDENTIFICATION OF NON-DERMATOPHYTIC MOULDS:

MACROSCOPIC APPEARANCE:

The fungi were identified based on the rate of growth, texture, pigment production and topography.

MICROSCOPIC EXAMINATION:

The microscopic features of the culture on LPCB mount and on slide culture were used to identify the species.

ANTIFUNGAL SUSCEPTIBILITY TESTING:

KIRBY-BAUER DISK DIFFUSION METHOD FOR *CANDIDA* SPECIES*:*

The antifungal susceptibility testing were done based on The Clinical Laboratory Standard Institute(CLSI) guidelines(M44-A2)[108].

REAGENTS:

Mueller-Hinton Agar

2% Glucose

0.5µg/ml methylene blue

Drugs-Fluconazole,Voriconazole.

CONTROL STRAIN:

Candida parapsilosis ATCC® 22019.

TURBIDITY STANDARD FOR INOCULUM PREPARATION

The inoculum density was standardized with 0.5Mc Farland standard.

INOCULUM PREPARATION

- All the *Candida* isolates were subcultured onto SDA and incubated at 37ºC overnight.
- Five distinct colonies were taken from the 24 hour old culture and suspended in 5ml of sterile saline(0.85%) .The resulting suspension was vortexed for 15 seconds .
- The inoculum density was adjusted to 0.5 Mc Farland standard at 530nm wavelength.
- This adjusted yeast suspension would yield $1x10^6$ to 5×10^6 cells/ mL.

INOCULATION ONTO TEST PLATES

- A sterile cotton swab was dipped into the yeast suspension and rotated several times, pressed against the wall of the tube firmly to remove the excess fluid from the swab.
- The swab was streaked over Mueller-Hinton Agar with 2% glucose and methylene blue.
- The antifungal disks were placed and plates were incubated at 37ºC for 24 hours.

INTERPRETATION OF RESULTS[108]:

The zone of inhibition was measured and interpreted as follows.

ANTIFUNGAL AGENT	DISK POTENCY	ZONE DIAMETER IN mm RANGES		
		R	S-DD	S
Fluconazole	25µg	≤14	15-18	≥19
Voriconazole	1µg	≤13	14-16	≥17

DETECTION OF MIC BY BROTH MICRODILUTION METHOD

The MIC for yeasts and moulds were determined based on CLSI guildeliness M27-A and M38-A2 respectively[123].

TEST STRAIN :

The MIC was determined for all yeasts , filamentous moulds and dermatophyte isolates .

REFERENCE STRAIN:

Candida parapsilosis ATCC® 22019 –For *Candida* species and dermatophytes.

Aspergillus flavus ATCC® 204304 – For filamentous fungi.

ANTIFUNGAL AGENTS USED:

Terbinafine, Itraconazole, Ketoconazole and Fluconazole were used for dermatophytes. Itraconazole, Ketoconazole, Fluconazole and Voriconazole were used for *Candida* species and filamentous fungi.

RANGE OF DRUG CONCENTRATIONS TESTED:

Terbinafine - 0.0313 to 16 µg/ml

Itraconazole - 0.0313 to 16 µg/ml

Ketoconazole - 0.0313 to 16 µg/ml

Voriconazole - 0.0313 to 16 µg/ml

Fluconazole - 0.125 to 64 µg/ml.

SOURCE OF DRUG:

All the drugs were obtained from The Global pharmacuetical pvt ltd,Chennai.

WEIGHING OF ANTIFUNGAL DRUGS

The weight of anti-fungal drugs were calculated according to the following formula.

$$\text{Weight(mg)} = \frac{\text{Volume(ml) X Concenteration(µg/ml)}}{\text{Assay Potency (µg/mg)}}$$

REAGENTS:

- A 2 day / 7 day old culture for inoculum preparation was used for *Candida* species and moulds respectively.
- Tween 20
- 0.85%Normal saline
- Buffer - MOPS (3-(N-morpholino) prophanesulfonic acid
- RPMI 1640 medium- (RoseWell Park Memorial Institute)
- with glutamine, without bicarbonate and with phenol red as indicator(Himedia,Mumbai,India.)

- 100% DMSO(dimethyl sulfoxide) was used to dissolve water insoluble drugs like terbinafine,itraconazole,ketoconazole and voriconazole.
- Sterile microtiter plate
- Distilled water was used to dissolve water soluble drug fluconazole.

INOCULUM PREPARATION

A) FILAMENTOUS FUNGI

1) The isolates were subcultured onto the Potato Dextrose Agar for 7 days to induce conidia formation.
2) The 7 day old colonies were then covered with 2ml of sterile 0.85%Normal Saline and a drop of TWEEN 20(0.01ml) was added.Then the colonies were gently probed with the pipette.
3) The resulting mixture of conidia was transferred into a sterile tube.The heavier particles were allowed to settle down for 3 to 5 mins.
4) The supernatant was transferred into a sterile tube ,cap tightened and mixed with vortex mixer for 15 seconds.
5) The resulting inoculum was read and adjusted to optical density of range 0.09 to 0.11(80 to 82% of transmittance) for *Aspergillus* species,*0.15 to 0.17 for Syncephalastrum and Fusarium* species,*0.25 to 0.3 for Bipolaris* species using a spectrophotometer .
6) The resulting suspension was further diluted 1:50 with the standard medium. So that the final inoculum would correspond to 0.4 X 10^4 to 5 X10^4 cfu/ml.

B) *CANDIDA* SPECIES

1) The test strains were subcultured onto SDA and incubated at 35ºC for 24 hours.
2) From the 24 hour old culture, 5 colonies were picked and suspended in 5ml of sterile normal saline(0.85%).
3) The suspension was vortexed for 15 seconds.
4) The resulting suspension was adjusted to 0.5 Mc Farland Standard at 530 nm wavelength with a spectrophotometer.
5) The resulting suspension was further diluted 1:50 with the standard medium so that the test inoculum was 1 X 10^3 to 5 X 10^3 CFU/ml.

DRUG POWDER PREPARATION:

- For water soluble drug fluconazole stock solution is prepared with sterile distilled water in the concentration of 5120 µg/ml.
- For water insoluble drug itraconazole,ketoconazole,voriconazole and terbinafine, stock solution is prepared with DMSO in the concenteration of 1600 µg/ml.

A)WATER INSOLUBLE DRUG:

DRUG SET PREPARATION:

Water insoluble drugs namely Itraconazole,Voriconazole,Ketoconazole and terbinafine were dissolved in DMSO and the intermediate drug concenterations were prepared as follows.

DRUG SET PREPARATION:

ROW 1 TUBES	1 (STOCK)	2	3	4	5	6	7	8	9	10
AMOUNT OF DMSO(ml)	-	0.5	1.5	3.5	0.5	1.5	3.5	0.5	1.5	3.5
AMOUNT OF DRUG(ml) (0.5ML)	-	FROM TUBE 1	FROM TUBE 1	FROM TUBE 1	FROM TUBE 4	FROM TUBE 4	FROM TUBE 4	FROM TUBE 7	FROM TUBE 7	FROM TUBE 7
INTERMEDIATE DRUG CONCENTERATION(µg/ml) IN ROW 1	1600	800	400	200	100	50	25	12.5	6.25	3.13

The final drug concentrations were prepared by diluting in RPMI as follows.

ROW 2 TUBES	1	2	3	4	5	6	7	8	9	10
AMOUNT OF RPMI IN ml	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
ADD DRUG FROM CORRESPONDING ROW(1) TUBES(1-10)(in µl)	100	100	100	100	100	100	100	100	100	100
FINAL DRUG CONCENTERATION(µg/ml) IN ROW (2) TUBES	32	16	8	4	2	1	0.5	0.25	0.125	0.0625

The finally diluted variable concenteration of drugs were added to the diluted inoculum as follows.

DRUG WITH INOCULUM PREPARATION:

MICROTITER PLATE WELLS (COLUMNS)	1	2	3	4	5	6	7	8	9	10
FROM ROW 2 TUBES ADD DRUG WITH RPMI TO MICROTITER PLATE(µl)	100	100	100	100	100	100	100	100	100	100
ADD INOCULUM TO PLATE (µl)	100	100	100	100	100	100	100	100	100	100
FINAL DRUG CONCENTRATION IN WELL(µg/ml)	16	8	4	2	1	0.5	0.25	0.125	0.062	0.0313

Growth control was added to column 11,with 100µl of RPMI and 100µl of inoculum.Drug control was added to column 12 with 100µl of RPMI and 100µl of drug.

INCUBATION:

The microtiter plates were incubated at 35°C for 24 hours for *Candida* species and 48 hours for filamentous fungi.

For dermatophytes the microtiter plates were incubated at 28ºC for 4 to 5 days[70],[77].

B)WATER SOLUBLE DRUG:

DRUG SET PREPARATION:

Stock solution was prepared by dissolving the water soluble drug fluconazole in distilled water.A serious of intermediate drug concenterations were prepared with RPMI as follows.

ROW 1 TUBES	1	2	3	4	5	6	7	8	9	10
AMOUNT OF RPMI (ml)	7	1	3	1	1.5	3.5	1	1.5	3.5	1
AMOUNT OF DRUG(ml)	FROM STOCK	FROM TUBE 1	FROM TUBE 1	FROM TUBE 3	FROM TUBE 3	FROM TUBE 3	FROM TUBE 6	FROM TUBE 6	FROM TUBE 6	FROM TUBE 9
	1	1	1	1	0.5	0.5	1	0.5	0.5	1
INTERMEDIATE DRUG CONCENTERATION(µg/ml) IN ROW 1	640	320	160	80	40	20	10	5	2.5	1.25

The final concenteration of drug was prepared from the intermediate drug concenteration by diluting in RPMI medium(1:50) as follows.

ROW 2 TUBES	1	2	3	4	5	6	7	8	9	10
AMOUNT OF RPMI IN ml	4	4	4	4	4	4	4	4	4	4
ADD DRUG FROM CORRESPONDING ROW 1 TUBES(1-10)(ml)	1	1	1	1	1	1	1	1	1	1
FINAL DRUG CONCENTERATION (µg/ml) IN ROW 2 TUBES	128	64	32	16	8	4	2	1	0.5	0.25

The finally diluted variable concentration of drugs were added to the diluted inoculum as follows.

DRUG WITH INOCULUM PREPARATION:

MICROTITER PLATE WELLS	1	2	3	4	5	6	7	8	9	10
FROM ROW 2 TUBES ADD DRUG TO MICROTITER PLATE(µl)	100	100	100	100	100	100	100	100	100	100
ADD INOCULUM TO PLATE (µl)	100	100	100	100	100	100	100	100	100	100
FINAL DRUG CONCENTRATION IN WELL(µg/ml)	64	32	16	8	4	2	1	0.5	0.25	0.125

Growth control was added to column 11,with 100µl of RPMI and 100µl of inoculum.Drug control was added to column 12 with 100µl of RPMI and 100µl of drug.

INCUBATION:

The microtiter plate was incubated at 35°C for 24 hours for *Candida* species and 48 hours for filamentous fungi.

For dermatophytes the microtiter plate was incubated at 28ºC for 4 to 5 days[70][77].

READNG OF RESULTS:

The growth in each of the microtiter well was compared with that of the growth control and scored as follows.

1) Optically clear absence of growth
2) Approximately 25% of the growth control or Slight growth.
3) Approximately 50% of the growth control or Prominent reduction in growth.
4) Approximately as of the growth medium or Slight reduction in the growth.
5) No reduction in the growth.

INTERPRETATION:

MIC for terbinafine was determined by noting 100% inhibition of growth control and that for azoles by noting 80% growth inhibition.

The MIC 50 and MIC 90 of the isolates were determined[118].

For *Candida* species[123] , the interpretation of MIC in (μg/ml) was as follows.

Drug	**Susceptible** (μg/ml)	**Susceptible-Dose Dependent** (μg/ml)	**Resistant** (μg/ml)
Fluconazole	≤8	16-32	≥64
Itraconazole	≤0.125	0.25-0.5	≥1
Voriconazole	≤1	2	≥4

STATISTICAL ANALYSIS:

Statistical analysis was done by using Statistical Package for Social Sciences(SPSS) version 21 .The test used in this study was Pearson's Chi Square analysis test . P value <0.05 is considered as statistically significant.

RESULTS

RESULTS

A total of 175 patients were included in the study and samples of nail scrapings were collected and processed. The details of the study population is as follows.

TABLE.1: AGE AND GENDER DISTRIBUTION (n=175)

AGE(YEARS)	MALE	FEMALE	TOTAL
21-30	2	-	2(1.14%)
31-40	10	7	17(9.72%)
41-50	21	31	52(29.72%)
51-60	27	35	62(35.43%)
61-70	24	9	33(18.85%)
71-80	7	2	9(5.14%)
TOTAL	91(52%)	84(48%)	175(100%)

Among the175 patients, majority of patients were in the age group of 51 – 60 years (35.43%) in both sexes. The mean age was observed to be 52.8 years .

The study population consisted of 52% males and 48% females in the ratio of 1.08 :1.

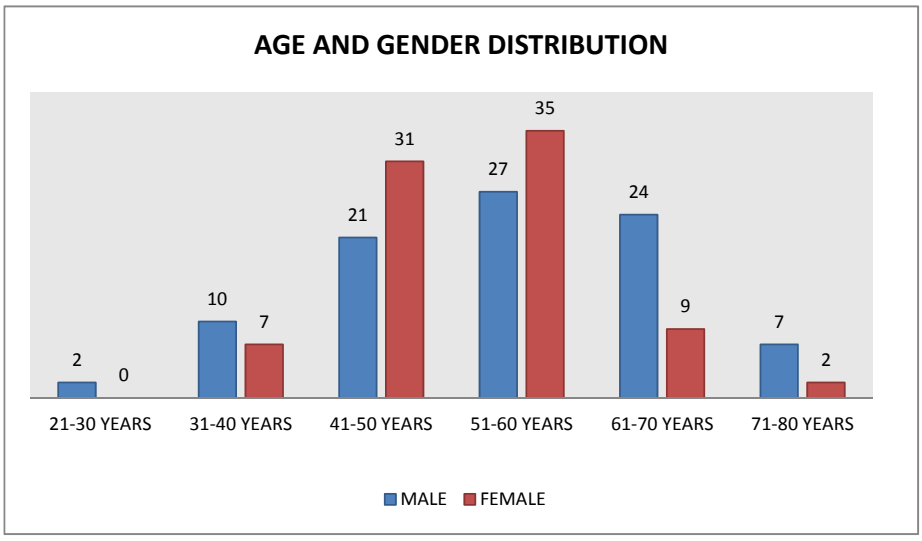


TABLE. 2 : DISTRIBUTION OF OCCUPATION (n=175)

OCCUPATION	MALE	FEMALE	TOTAL	PERCENTAGE
FARMERS	52	25	77	44
HOUSEWIVES	NA	46	46	26.28
CARPENTER	7	0	7	4
DRIVER	**7**	0	7	4
WATCHMAN	7	0	7	4
SHOPKEEPER	5	1	6	3.42
TAILOR	5	1	6	3.42
CLERICAL STAFF	3	3	6	3.42
ENGINEER	4	2	6	3.42
STAFF NURSE	0	5	5	2.9
SWEEPER	1	1	2	1.14
TOTAL	91	84	175	100

NA-Not applicable.

Among the study population, 44% of them were farmers and 26.28% were housewives.

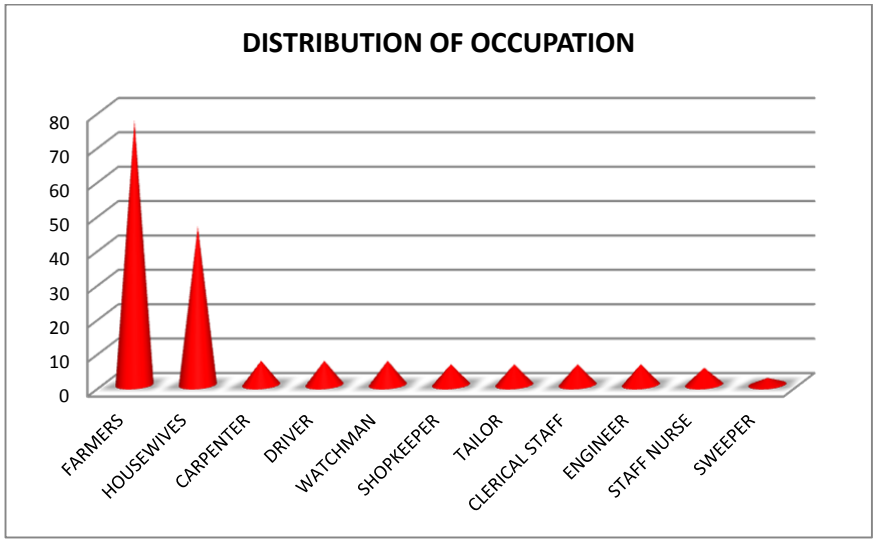


TABLE.3: DISTRIBUTION OF TYPE OF DIABETES MELLITUS IN STUDY POPULATION(n=175)

TYPE OF DIABETES	NO.OF.PATIENTS	PERCENTAGE
TYPE I	14	8
TYPE II	161	92
TOTAL	175	100

Among the patients with onychomycosis,92% had type II diabetes mellitus (n=161) and 8% had type I diabetes mellitus (n=14).

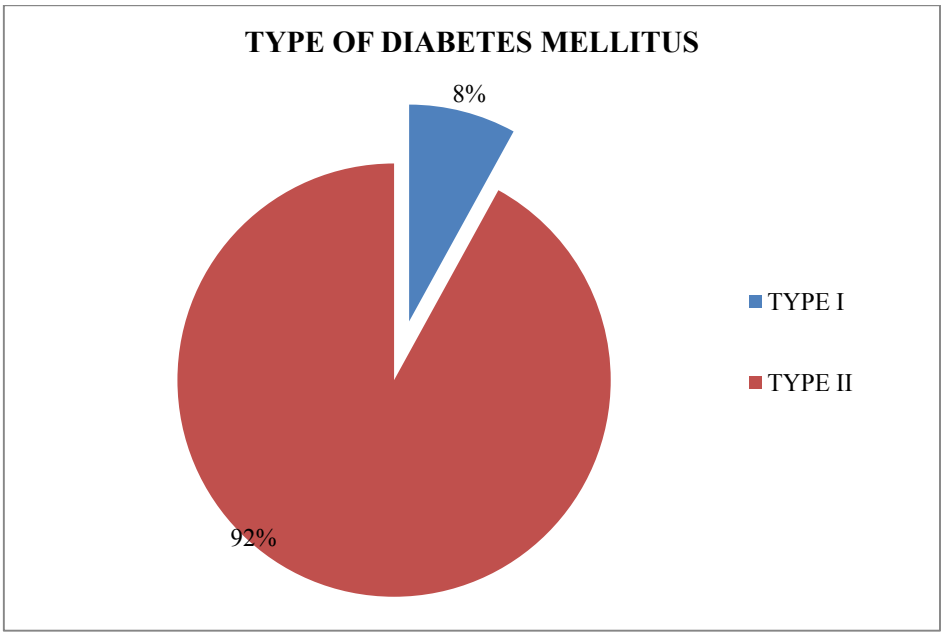


TABLE.4: DISTRIBUTION OF CLINICAL PATTERN OF ONYCHOMYCOSIS(n=175)

CLINICAL TYPE	FINGER NAIL	TOE NAIL	BOTH NAIL	TOTAL
DLSO	25	109	22	156(89.5%)
PSO	1	0	1	2(1.14%)
TDO	0	10	7	17(9.7%)
TOTAL	26(15%)	119(68%)	30(17%)	175(100%)

Among 175 patients, 156 (89.5%) had DLSO (Distal lateral subungual onychomycosis). TDO (Total Dystrophic Onychomycosis)was present in 17(9.7%) and PSO(Proximal Subungual Onychomycosis) in 2 (1.14%) patients.

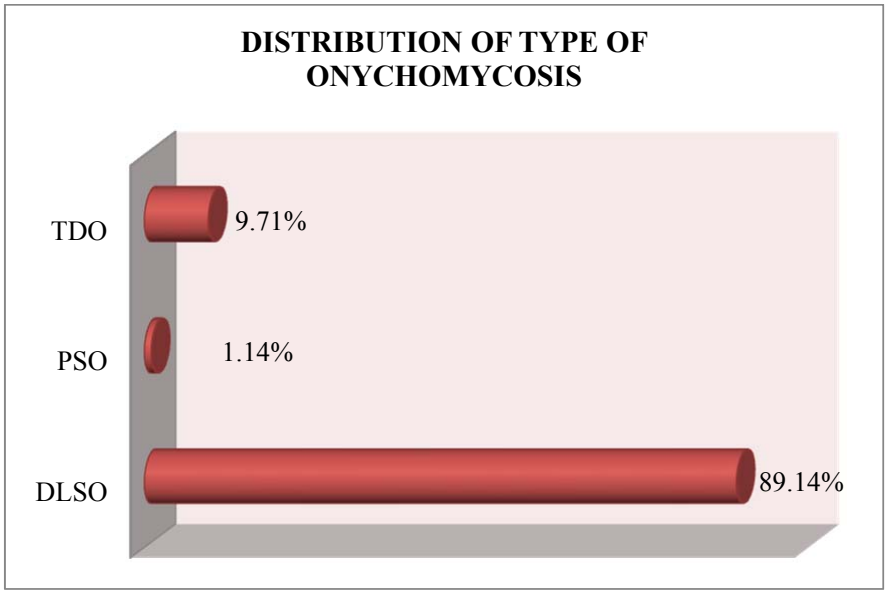


TABLE.5 : DETAILS OF SITE OF ONYCHOMYCOSIS(n=175)

SITE	MALE	FEMALE	TOTAL
FINGER	13	13	26(15%)
TOE	62	57	119(68%)
BOTH	16	14	30(17%)
TOTAL	91	84	175(100%)

Toe nails were predominantly involved in 68% of cases (n= 119) in both sexes while finger nails were involved in 15% of patients (n=26) . Both toe and finger nails were affected in 17% of patients(n=30) .

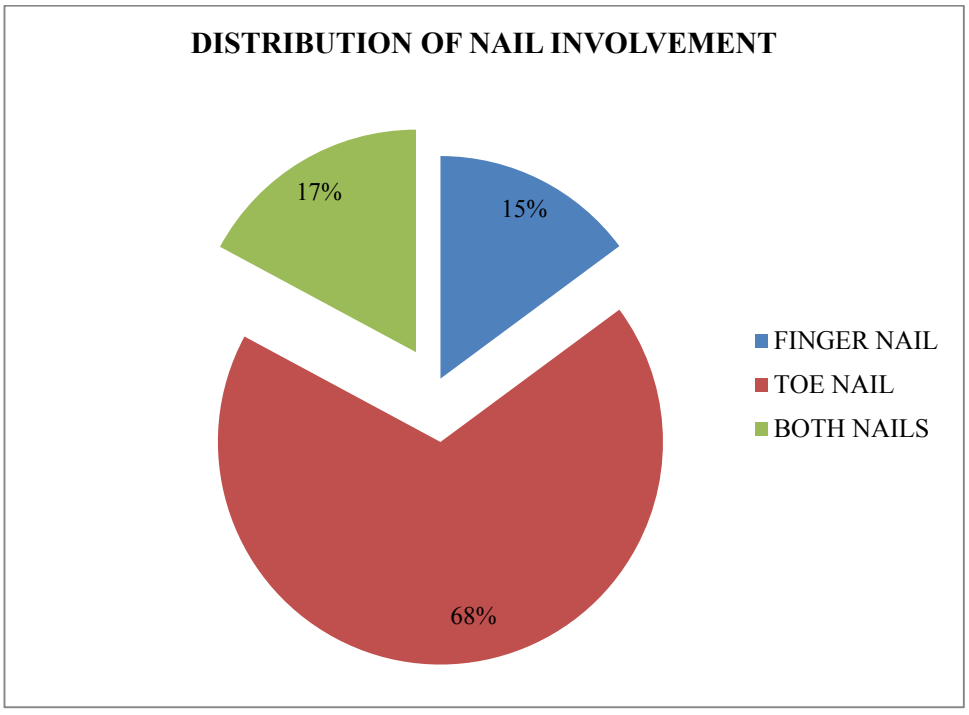


TABLE.6: DURATION OF ONYCHOMYCOSIS(n=175)

DURATION	MALE	FEMALE	TOTAL	PERCENTAGE
Upto 1year	32	22	54	30.86%
>1year-5year	47	45	92	52.57%
>5year	12	17	29	16.57%
TOTAL	91	84	175	100%

Majority of the patients, (52.57%) gave history of nail involvement for a duration of 1 to 5years (n=92).30.86% had nail affliction for less than 1year(n=54) and 16.57 % had a duration of more than 5 years (n=29) .

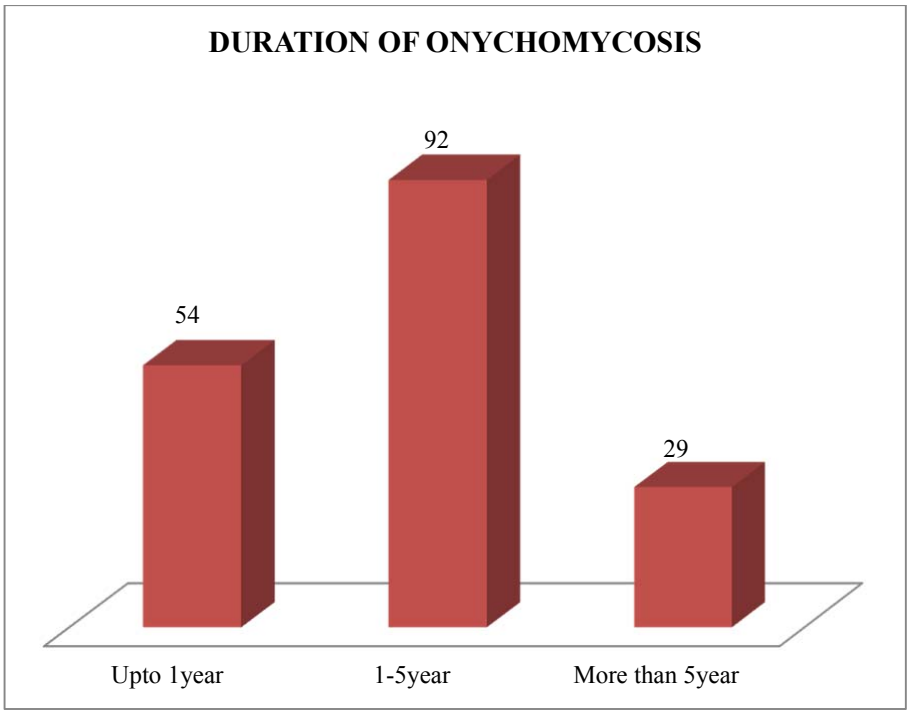


TABLE.7 : SEVERITY OF ONYCHOMYCOSIS(n=175)

DEGREE	TOTAL	PERCENTAGE
MILD	152	87%
MODERATE	5	3%
SEVERE	18	10%
TOTAL	175	100%

Among the study population,87%(n=152) of the patients had mild onychomycosis. 10%(n=18) of patients had severe onychomycosis and 3%(n=5) had moderate degree of onychomycosis.

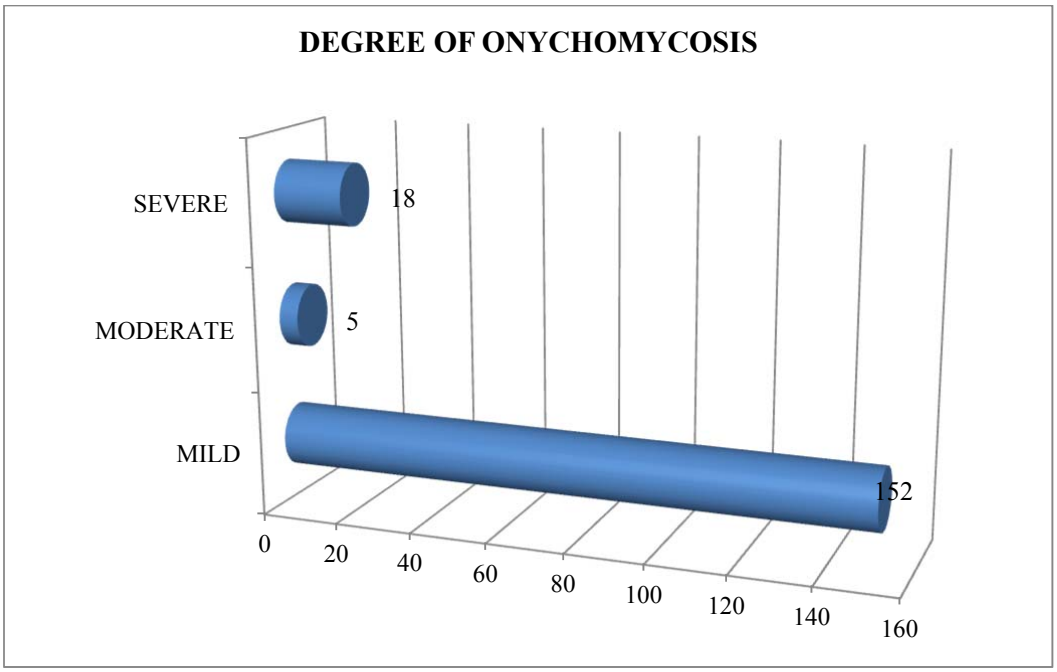


TABLE.8: CORRELATION OF SEVERITY OF ONYCHOMYCOSIS WITH TYPE OF DIABETES MELLITUS(n=175).

TYPE OF DM	MILD	MODERATE	SEVERE	TOTAL
Type I	13(7.5%)	0	1(0.5%)	14(8%)
Type II	139(79.5%)	5(3%)	17(9.5%)	161(92%)
TOTAL	152(87%)	5(3%)	18(10%)	175(100%)

Mild and severe onychomycosis were present in 79.5% and 9.5% of type II diabetes mellitus patients respectively.There was no statistical significance between the severity of onychomycosis and type of diabetes mellitus. (p=0.725).

CORRELATION OF SEVERITY OF ONYCHOMYCOSIS WITH TYPE OF DIABETES MELLITUS(n=175)

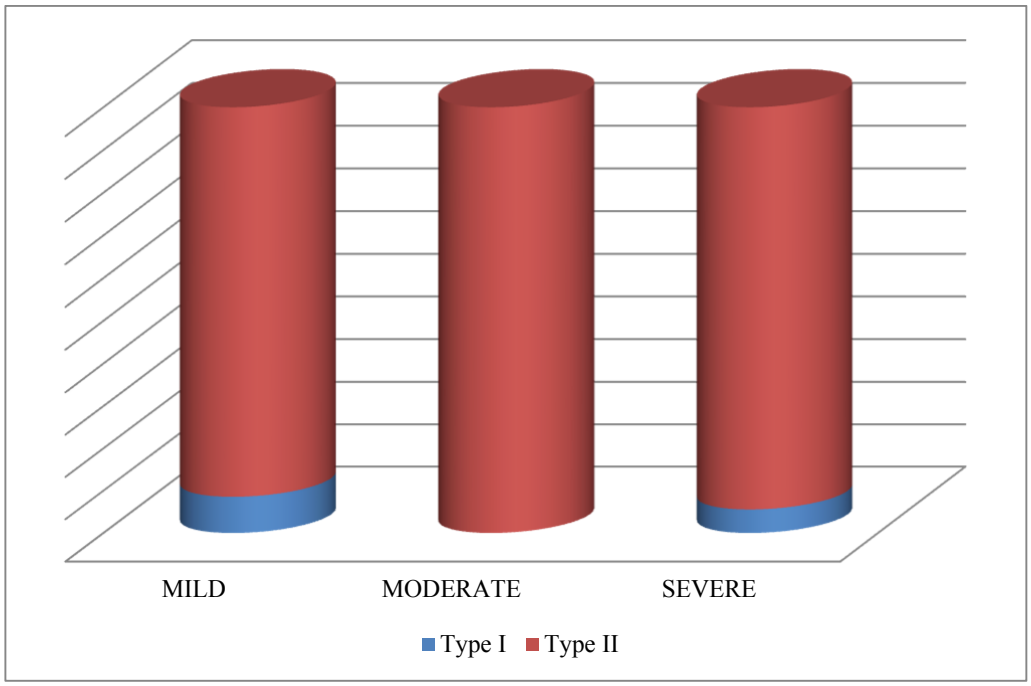


TABLE.9 : CORRELATION OF SEVERITY OF ONYCHOMYCOSIS WITH DURATION OF DIABETES MELLITUS (n=175)

DURATION	MILD	MODERATE	SEVERE	TOTAL
Less than 1 year	32(18.3%)	0	5(2.9%)	37(21.2%)
1-5 years	54(30.9%)	3(1.7%)	4(2.3%)	61(34.9%)
5-10years	51(29.2%)	2(1.3%)	8(4.4%)	61(34.9%)
10-15years	15(8.6%)	0	1(0.4%)	16(9%)
TOTAL	152(87%)	5(3%)	18(10%)	175(100%)

Majority of the patients had a history of diabetes mellitus for a duration of 1 to 5 years (34.9%) and 5 to 10 years (34.9%). Among 18 patients with severe onychomycosis,4.4% (n=8) had diabetes mellitus for a duration of 5-10 years.There was no statistical significance between the severity of onychomycosis and the duration of diabetes mellitus(p=0.600).

CORRELATION OF SEVERITY OF ONYCHOMYCOSIS WITH DURATION OF DIABETES MELLITUS(n=175)

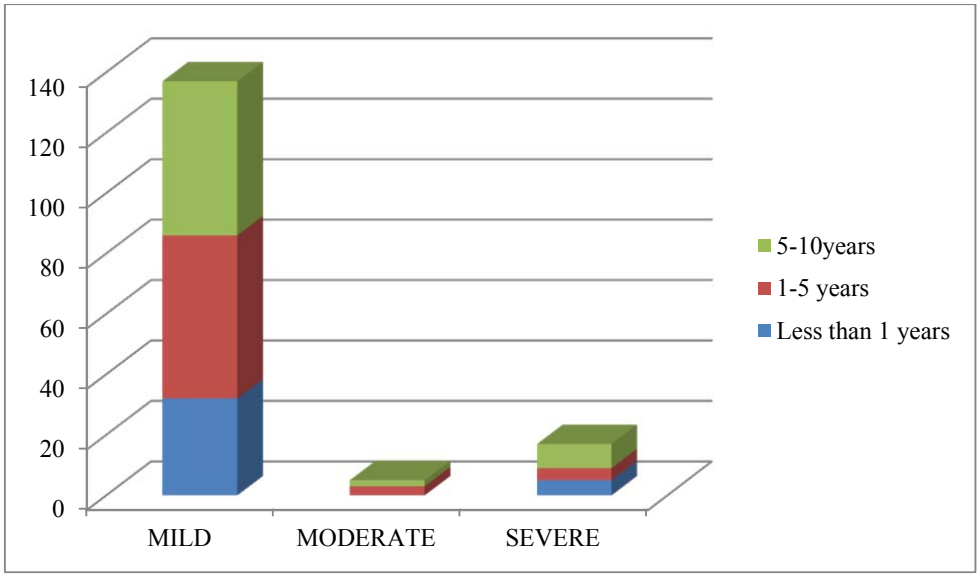


TABLE.10 : CORRELATION OF SEVERITY OF ONYCHOMYCOSIS WITH BLOOD SUGAR LEVELS (n=175)

BLOOD SUGAR LEVELS (mg/dl)	MILD	MODERATE	SEVERE	TOTAL
100-200	131 (75.05%)	5 (3%)	17 (9.6%)	153 (87.65%)
201-300	16 (8.95%)	0	1 (0.4%)	17 (9.35%)
301-400	5 (3%)	0	0	5 (3%)
TOTAL	152(87%)	5(3%)	18(10%)	175 (100%)

Most of the patients(87.65%) had blood sugar levels in the range of 100 to 200mg/dl and among them 75.05% had mild degree of onychomycosis. There was no statistical significance between the severity of onychomycosis and the blood sugar level (p=0.679).

CORRELATION OF SEVERITY OF ONYCHOMYCOSIS WITH BLOOD SUGAR LEVELS(n=175)

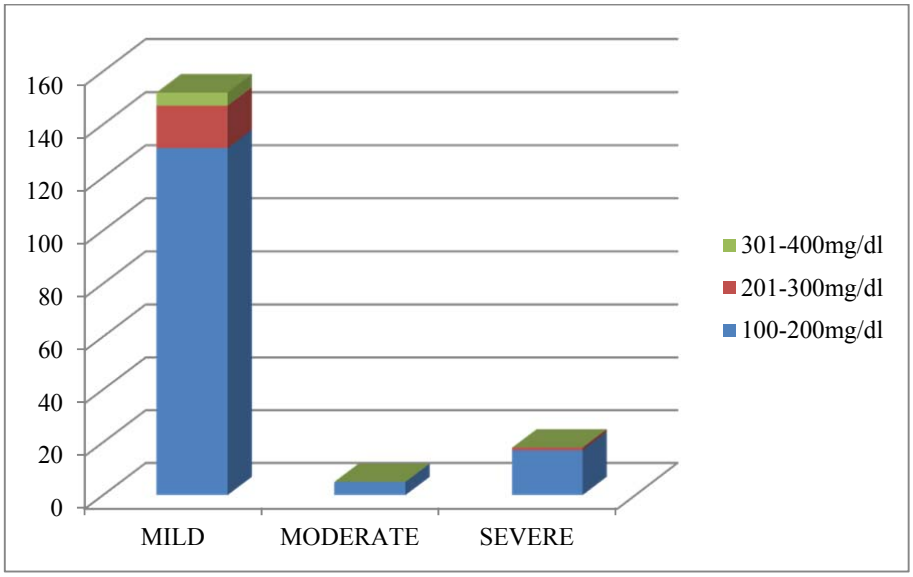


TABLE.11: RESULTS OF CULTURE AND KOH MOUNT(n=175)

METHOD	POSITIVE	NEGATIVE
KOH mount	123(70.29%)	52(29.71%)
CULTURE	81(46.28%)	94(53.72%)

The culture positivity rate was 46.28% and the rate of detection by KOH mount was 70.29%.

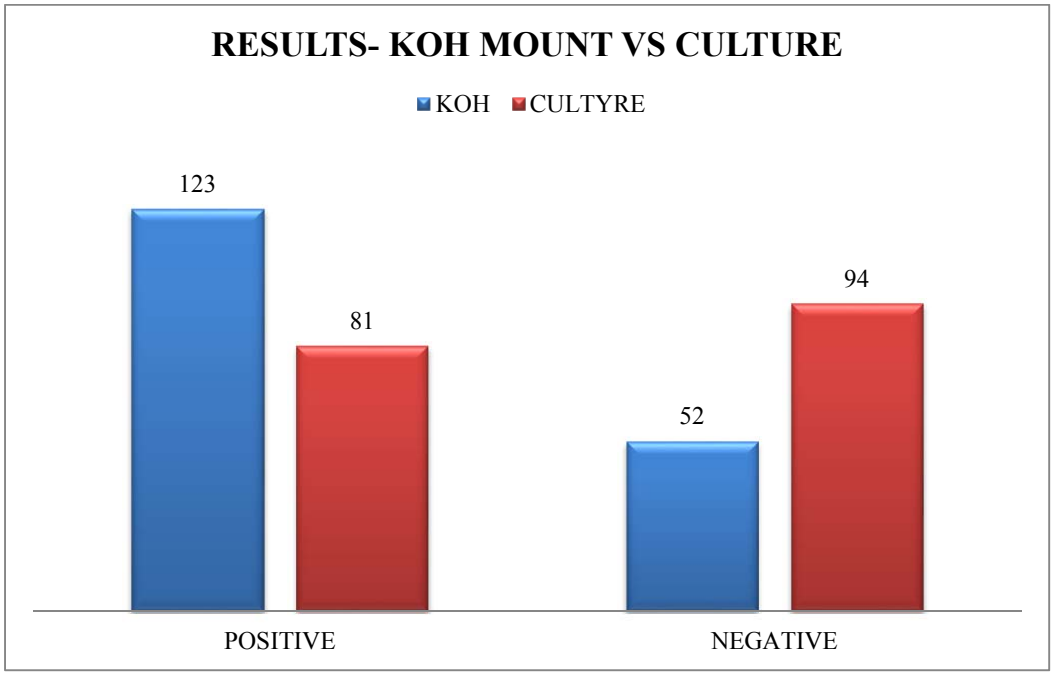


TABLE.12: EVALUATION OF CULTURE AND KOH(n=175)

KOH POSITIVE CULTURE POSITIVE	KOH NEGATIVE CULTURE POSITIVE	KOH POSITIVE CULTURE NEGATIVE	KOH NEGATIVE CULTURE NEGATIVE
77(44%)	4(2.29%)	46(26.29%)	48(27.42%)

Among 175 patients, 4 patients (2.29%) were culture positive, but potassium hydroxide mount negative and 26.29% of patients were potassium hydroxide mount positive but culture negative.

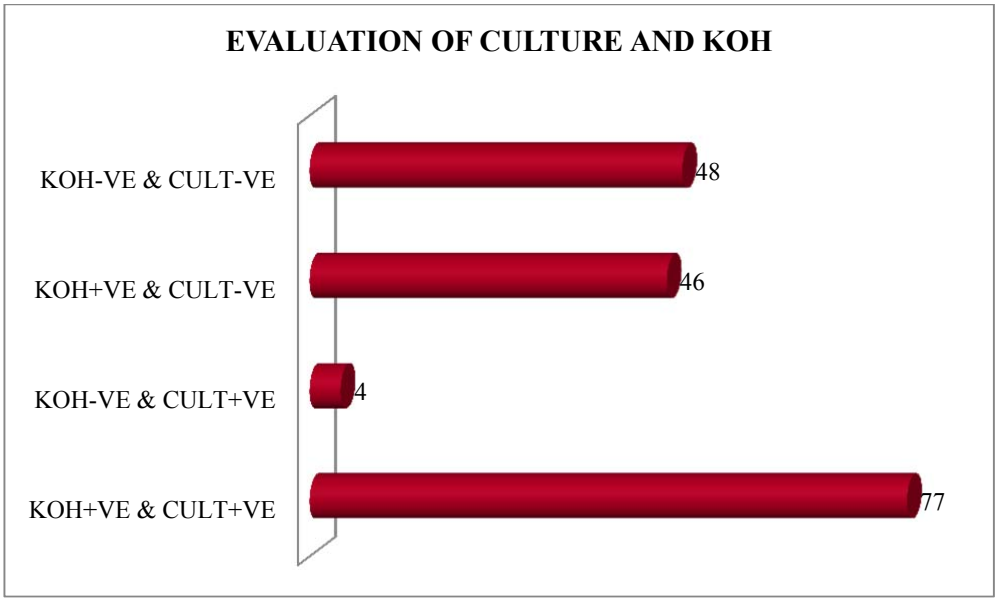


TABLE.13: DISTRIBUTION OF FUNGAL ISOLATES:(n=81)

ISOLATES	DLSO	TDO	TOTAL	PERCENTAGE
DERMATOPHYTES	18	3	21	25.92%
CANDIDA SPECIES	30	2	32	39.50%
NON-DERMATOPHYTIC MOULDS	26	2	28	34.58%
TOTAL	74 91.35%	7 8.65%	81	100%

Candida species was the most common isolate(39.50%),followed by non-dermatophytic moulds (34.58%) and dermatophytes (25.92%). Majority of the isolates (91.35%)were associated with DLSO type of onychomycosis.

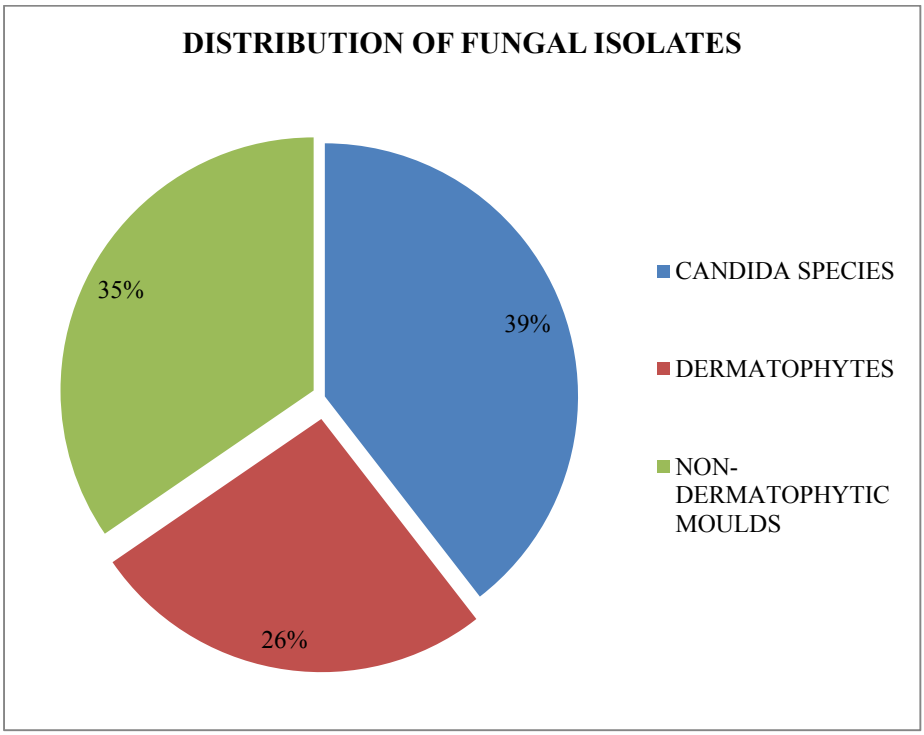


TABLE.14: DISTRIBUTION OF *CANDIDA* ISOLATES(n=32)

ISOLATES	NUMBER	PERCENTAGE
C.albicans	8	25%
C.parapsilosis	20	62.5%
C.tropicalis	4	12.5%
TOTAL	32	100%

Among the *Candida* species ,*C.parapsilosis*(62.5%) was the most common species followed by *C.albicans*(25%) and *C.tropicalis*(12.5%).

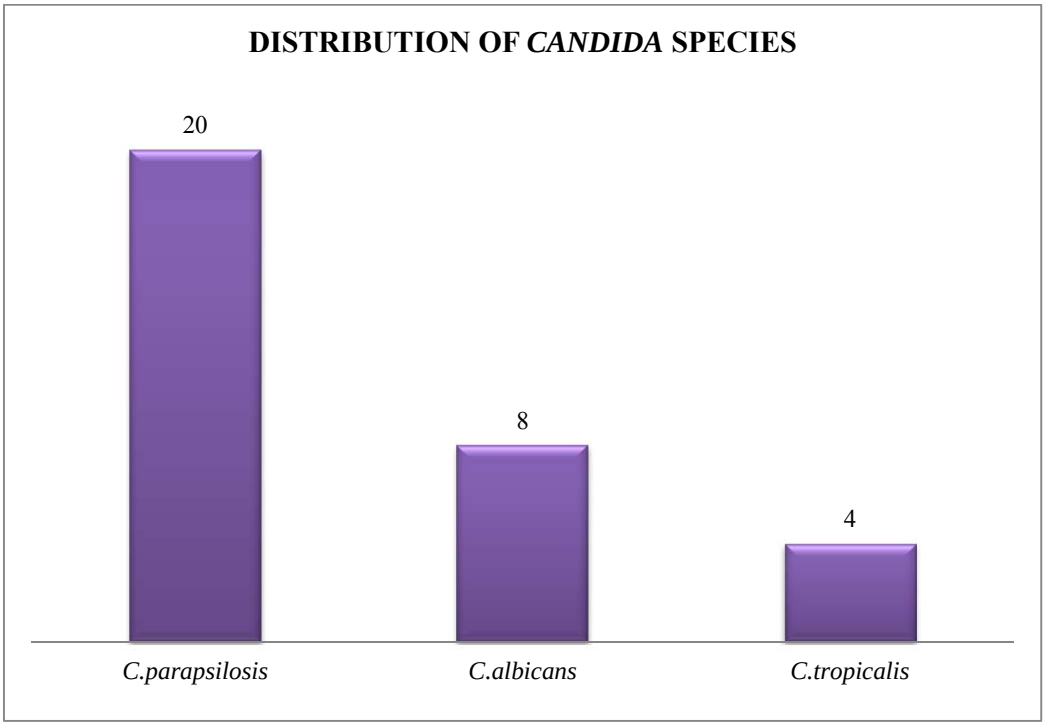


TABLE 15: EPIDEMIOLOGICAL PATTERN IN CANDIDIAL ONYCHOMYCOSIS:(n=32)

EPEDIMEMIOLOGICAL VARIABLE	NUMBER		PERCENTAGE
AGE	30 to 50 years	13	40.62%
	Above 50 years	19	59.38%
SEX	Male	11	34.38%
	Female	21	65.62%
OCCUPATION	Farmer	12	37.5%
	Housewives	10	31.25%
	Watchman	3	9.38%
	Clerical staff	2	6.25%
	Driver	2	6.25%
	Others	3	9.37%
NAIL INVOLVEMENT	Finger nail	14	43.75%
	Toe nail	13	40.62%
	Both nails	5	15.63%
TYPE	DLSO	30	93.75%
	TDO	2	6.25%
OTHER COMORBID CONDITIONS	Hypertension	7	21.88%
	H/O trauma	4	12.5%
	H/o frequent handwashing	2	6.25%

The frequency of *Candida* species was more common among the age group above 50years(59.38%) and in females(65.62%). 37.5% were farmers and 31.25% were housewives. Finger nails were involved in 43.75% of patients. Among the patients with candidal onychomycosis ,93.75% of patients had DLSO type of onychomycosis. 21.88% of patients had hypertension.There was H/O trauma in 12.5% of patients.

TABLE.16: DISTRIBUTION OF NON-DERMATOPHYTIC MOULDS(n=28).

ISOLATES	TOTAL	PERCENTAGE
A.niger	3	10.71%
A.fumigatus	4	14.27%
Fusarium species	16	57.14%
Syncephalastrum species	1	3.58%
Penicillium species	1	3.58%
Absidia species	1	3.58%
Bipolaris species	2	7.14%
TOTAL	28	100%

Among non-dermatophytic moulds, *Fusarium* species was the most common etiological agent (57.14%), followed by *Aspergillus fumigates* (14.27%), *Aspergillus niger* (10.71%), *Bipolaris* species (7.14%), *Absidia* species (3.58%) ,*Penicillium* species*(*3.58%) and *Syncephalastrum* species (3.58%).

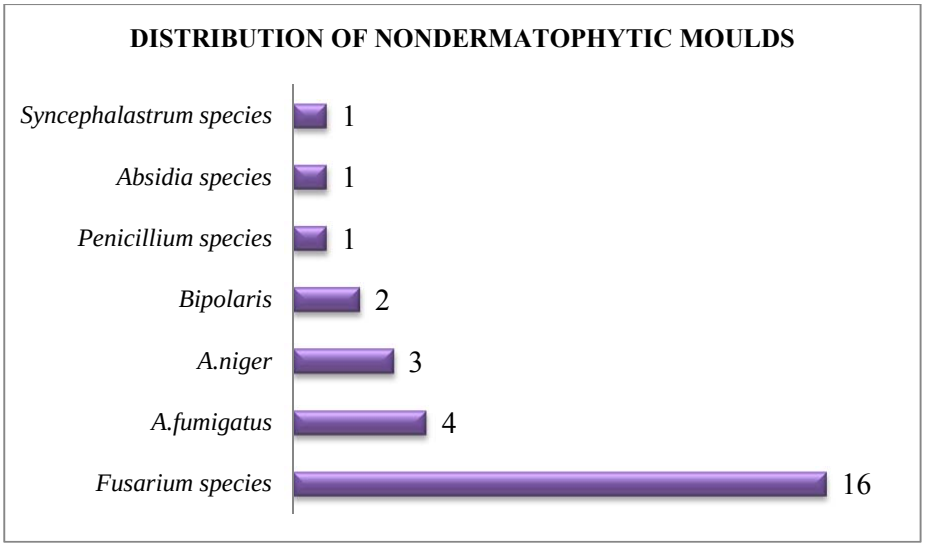


TABLE.17: EPIDEMIOLOGICAL PATTERN IN NON-DERMATOPHYTIC ONYCHOMYCOSIS (n=28)

EPEDIMEMIOLOGICAL VARIABLE	NUMBER		PERCENTAGE
AGE	35 to 50 years	11	39.28%
	Above 50 years	17	60.72%
SEX	Male	17	60.72%
	Female	11	39.28%
OCCUPATION	Farmer	14	50%
	Housewives	4	14.28%
	Tailor	4	14.28%
	Carpenter	2	7.14%
	Driver	2	7.14%
	Others	2	7.14%
NAIL INVOLVEMENT	Finger nail	3	10.72%
	Toe nail	21	75%
	Both nails	4	14.28%
TYPE	DLSO	26	92.86%
	TDO	2	7.14%
OTHER COMORBID CONDITIONS	Hypertension	3	10.72%
	H/O trauma	6	21.43%
	CKD	2	7.14%
	H/o frequent handwashing	2	7.14%

The nondermatophytic moulds were more common among the age group above 50years (60.72%) and in males (60.72%). 50 % of the patients were farmers and 14.28% were housewives. Toe nails were involved in 75 % of patients. Among the patients with non-dermatophytic mould infection, 92.86% of patients had DLSO type of onychomycosis. 21.43% of patients had trauma and 10.72% of the patients had hypertension.

TABLE.18:DISTRIBUTION OF DERMATOPHYTE ISOLATES IN STDUY POPULATION (n=21)

ISOLATE	TOTAL	PERCENTAGE
T.rubrum	13	61.90%
T.tonsurans	6	28.58%
E.floccosum	1	4.76%
M.gypseum	1	4.76%
TOTAL	21	100%

Among the dermatophytes, the most common isolate was *Trichophyton rubrum* (61.90%), followed by *Trichophyton tonsurans* (28.58%), *Epidermophyton floccosum* (4.76%) and *Microsporum gypseum* (4.76%).

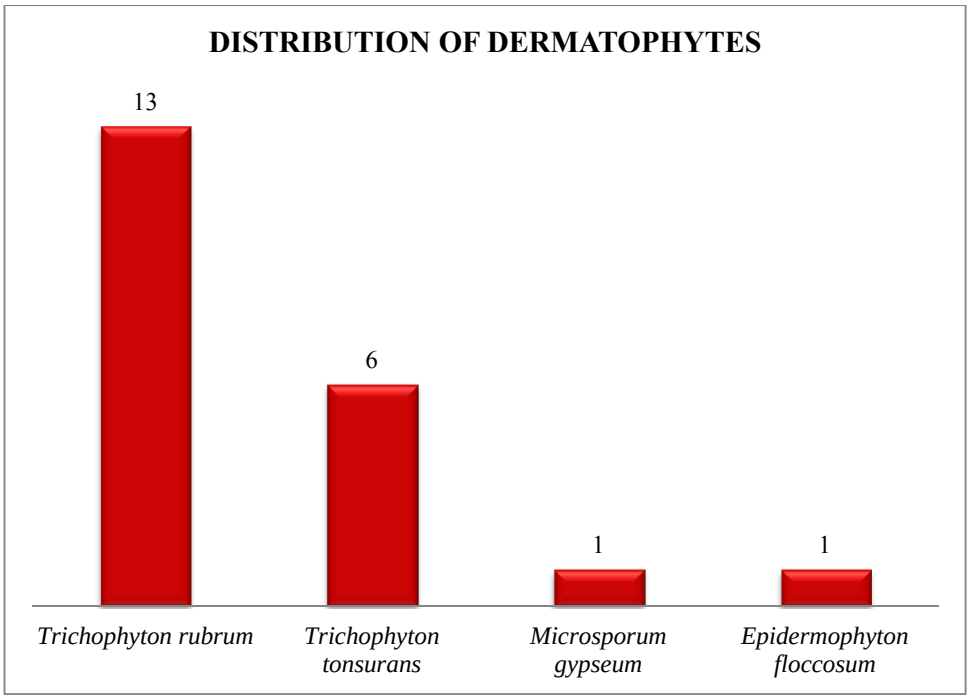


TABLE.19:DISTRIBUTION OF DERMATOPHYTES IN EXTRAUNGUAL SITES

ISOLATE	Trunk /Face/head	Groin	Feet	Hand	NO.OF ISOLATES
Trichophyton rubrum(n=13)	5 (38.46%)	1 (16.66%)	0	0	5 (38.46%)
Trichophyton tonsurans(n=6)	1 (16.66%)	1 (16.66%)	2 (33.33%)	0	5 (83.33%)
Microsporum gypseum(n=1)	0	0	0	0	0
Epidermophyton floccosum(n=1)	1 (100%)	1 (100%)	0	0	1 (100%)

Tinea corporis was found to be associated with 38.46% of *T.rubrum* infection. Tinea pedis was found to be the associated with 33.33% of *T.tonsurans*. The single isolate of *Epidermophyton floccosum* was found to be associated with both tinea corporis and tinea cruris.

TABLE.20:SUSCEPTIBILITY PATTERN OF *CANDIDA* ISOLATES BY DISK DIFFUSION METHOD

ISOLATE	NO OF ISOLATES	FLUCONAZOLE	VORICONAZOLE
C.parapsilosis	20	20(100%)	20(100%)
C.albicans	8	8(100%)	8(100%)
C.tropicalis	4	4(100%)	4(100%)

All the *Candida* species were susceptible to both fluconazole and voriconazole.

TABLE 21 : MIC OF *CANDIDA* SPECIES BY MICROBROTH DILUTION METHOD

***CANDIDA* SPECIES**	**No**	**FLU (64-.125) µg/ml**		**KET (16-.031) µg/ml**		**ITRA (16-0.0313) µg/ml**		**VORI (16-0.0313) µg/ml**	
		MIC_{50}	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
C.parapsilosis	20	1	2	0.5	1	0.062	0.125	0.0313	0.0313
C.albicans	8	1	2	0.5	1	0.062	0.125	0.0313	0.0313
C.tropicalis	4	1	2	0.5	1	0.062	0.125	0.0313	0.0313

FLU-Fluconazole,KET–Ketoconazole,ITRA–Itraconazole,VORI- Voriconazole

The MIC of *Candida* species were within the following range as follows: Fluconazole: 1- 2 µg/ml, Ketoconazole:0.5-1 µg/ml,

Itraconazole:0.0625 – 0.125 µg/ml and Voriconazole:0.0313 µg/ml .

All of the isolated *Candida* speciess were sensitive to fluconazole,itraconazole and voriconazole by MIC determination.

TABLE.22:ANTIFUNGAL SUSCEPTIBILITY PATTERN OF NON-DERMATOPHYTIC MOULDS

ISOLATES	No	FLU (64-0.125) µg/ml		KET (16-0.0313) µg/ml		ITRA (16-0.0313) µg/ml		VORI (16 - 0.0313) µg/ml	
		MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
Absidia species	1	16	16	8	8	4	4	1	1
Syncephalastrum species	1	4	4	0.5	0.5	0.0625	0.0625	0.0313	0.0313
Penicillium species	1	1	1	8	8	1	1	0.0313	0.0313
Bipolaris species	2	8	8	2	4	1	1	0.0625	0.0625
A.niger	3	4	8	1	2	0.5	1	0.0313	0.0313
A.fumigatus	4	16	32	0.5	2	0.5	0.5	0.0313	0.0313
Fusarium species	16	32	64	16	32	8	16	4	8

FLU-Fluconazole,KET–Ketoconazole,ITRA–Itraconazole,VORI-Voriconazole.

The MIC range of the various non-dermatophytic moulds were as follows:

Fluconazole: 1 -64 µg/ml, Ketoconazole: 0.5 - 32 µg/ml

Itraconazole: 0.0625- 16 µg/ml and Voriconazole: 0.0313– 8 µg/ml.

TABLE.23:ANTIFUNGAL SUSCEPTIBILITY PATTERN OF DERMATOPHYTES

ISOLATES	No	FLU (64-0.125) µg/ml		KET (16 -0.0313) µg/ml		ITRA (16 -0.0313) µg/ml		TERBI (16 -0.0313) µg/ml	
		MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
Trichophyton rubrum	13	8	32	0.5	2	0.25	0.5	0.0313	0.0313
Trichophyton tonsurans	6	4	8	0.5	2	0.25	1	0.0313	0.0313
Microsporum gypseum	1	8	8	2	2	1	1	0.0313	0.0313
Epidermophyton floccosum	1	16	16	1	1	0.5	0.5	0.0313	0.0313

FLU-Fluconazole, KET – Ketoconazole, ITRA – Itraconazole,TERBI- Terbinafine.

The MIC range of dermatophytes were as follows:

Fluconazole:4-32µg/ml

Ketoconazole: 0.5-2µg/ml

Itraconazole:0.25-1µg/ml and

Terbinafine:0.0313µg/ml .

COLOUR PLATES

CLINICAL PRESENTATION

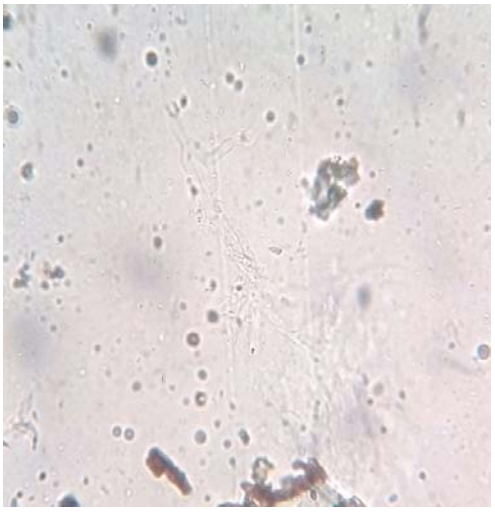
TOE NAIL ONYCHOMYCOSIS - DLSO

FINGER NAIL ONYCHOMYCOSIS

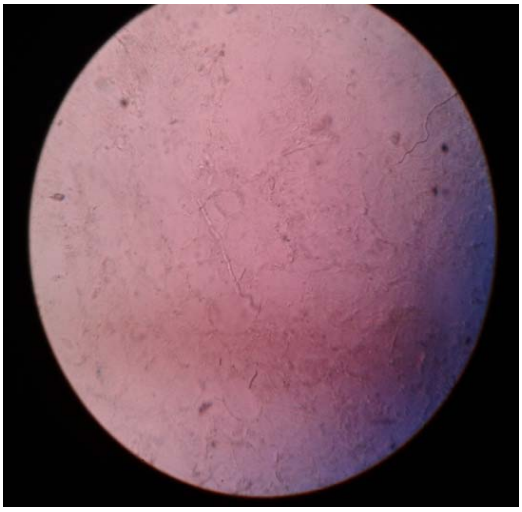
- INVOLVING ALL 10 NAILS

KOH MOUNT

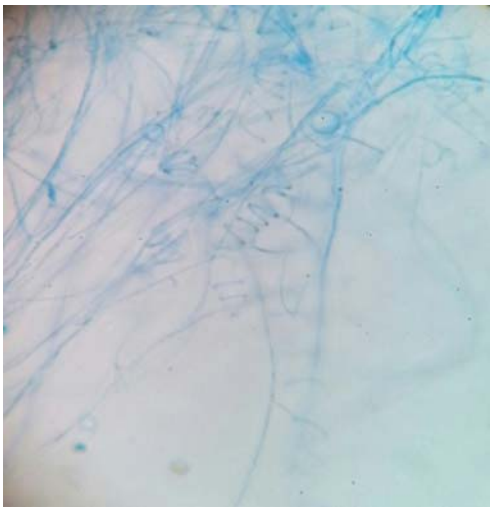
HYALINE ASEPTATE HYPHAE



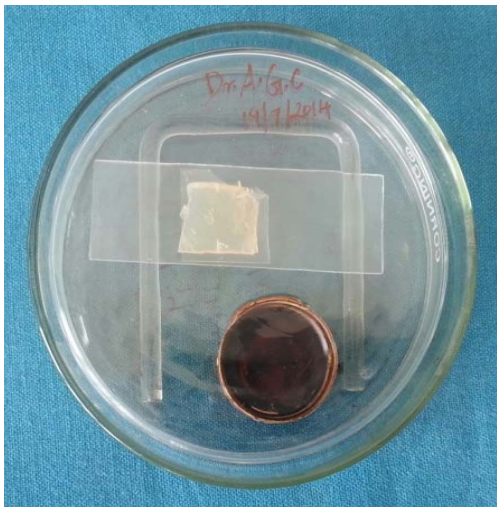
ARTHROCONIDIA



SPIRAL HYHAE



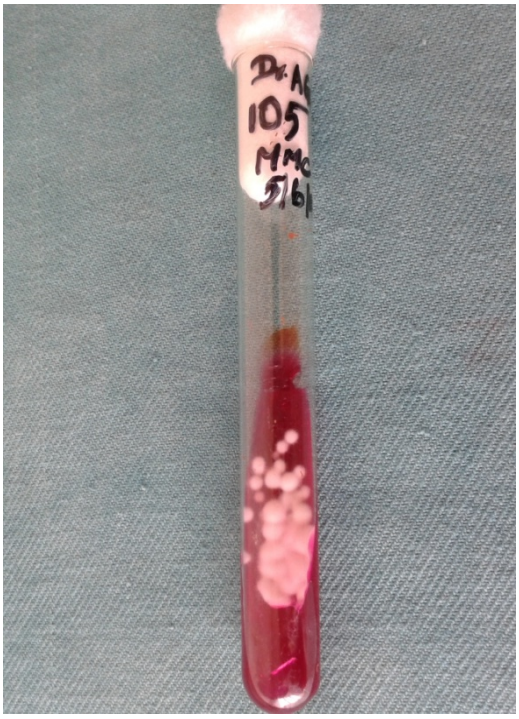
SLIDE CULTURE SET



DERMATOPHYTE TEST MEDIUM

UNINOCULATED MEDIUM

POSITIVE

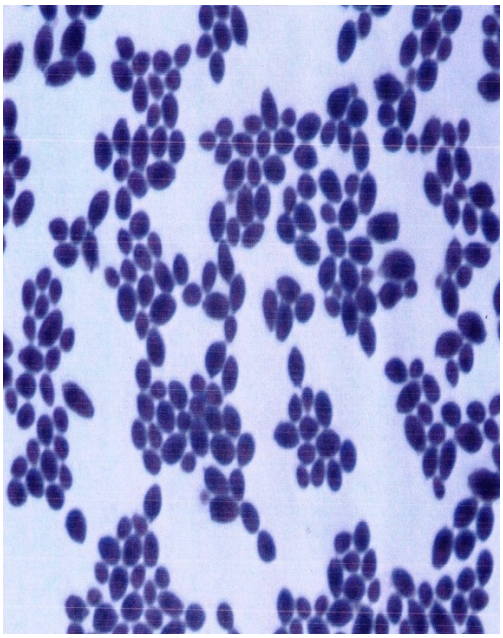


CANDIDA SPECIES

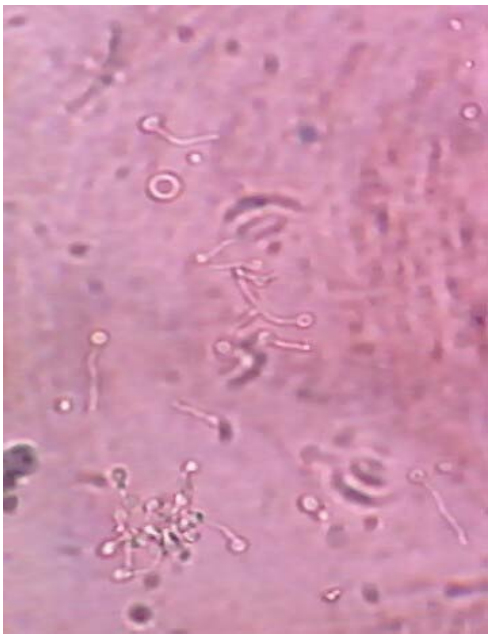
OBVERSE

REVERSE

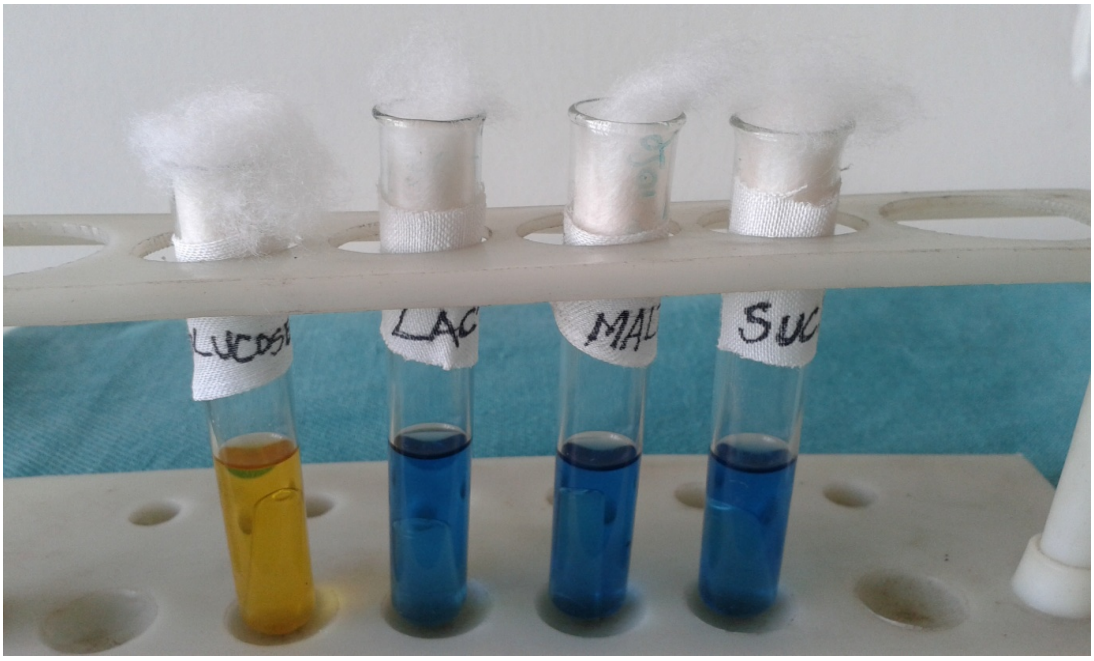
GRAM STAIN



GERM TUBE TEST



SUGAR FERMENTATION TEST – *C.parapsilosis*

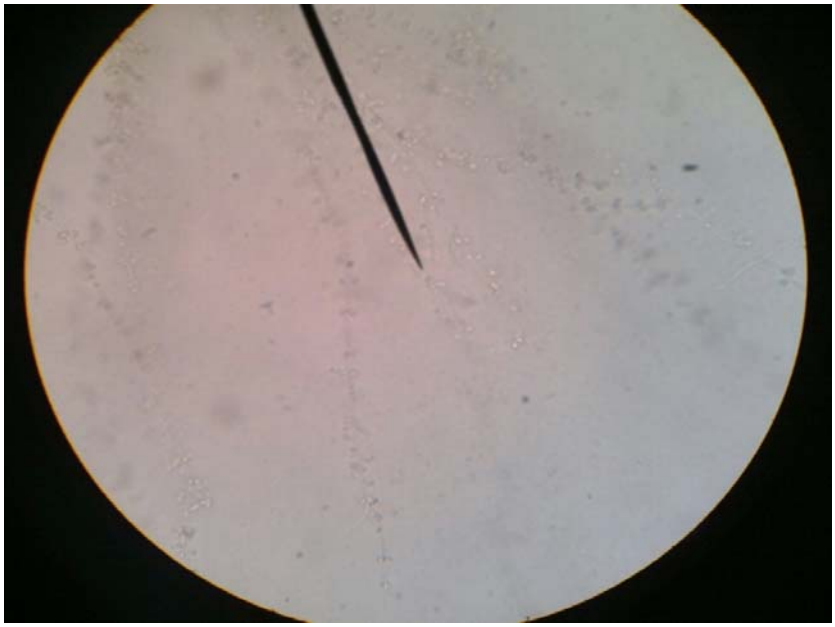


SUGAR ASSIMILATION TEST- *C.albicans*

CHROM AGAR CANDIDA MEDIUM

CORN MEAL AGAR-*C.tropicalis*

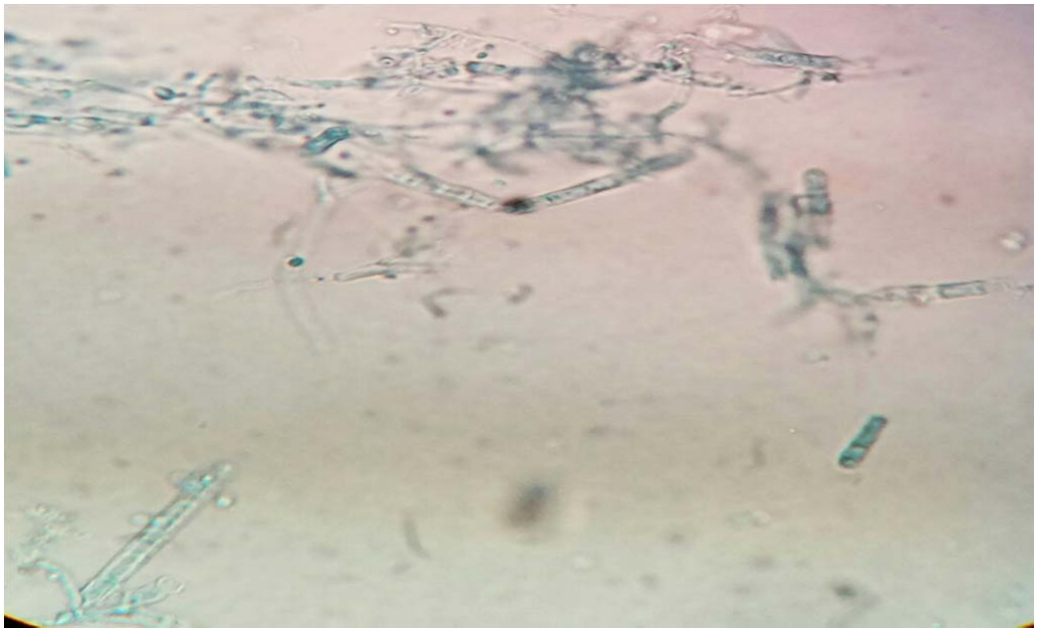
Blastoconidia borne singly and arranged irregularly on pseudohyphae



MACROSCOPIC APPEARANCE OF
Trichophyton rubrum
OBVERSE –powdery. REVERSE (RED PIGMENTATION)

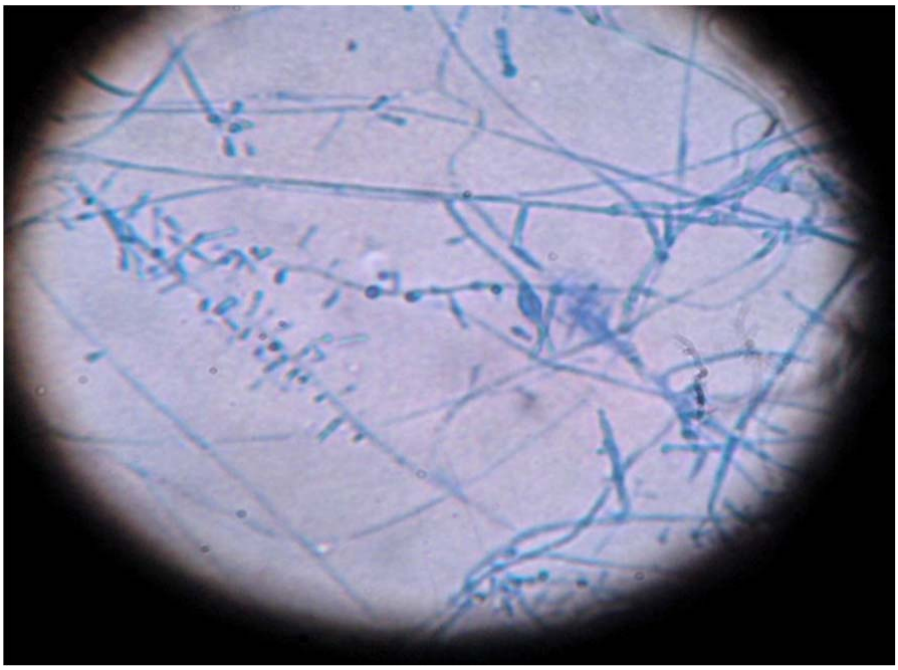
MICROSCOPIC APPEARANCE

- Pencil Shaped Macroconidia

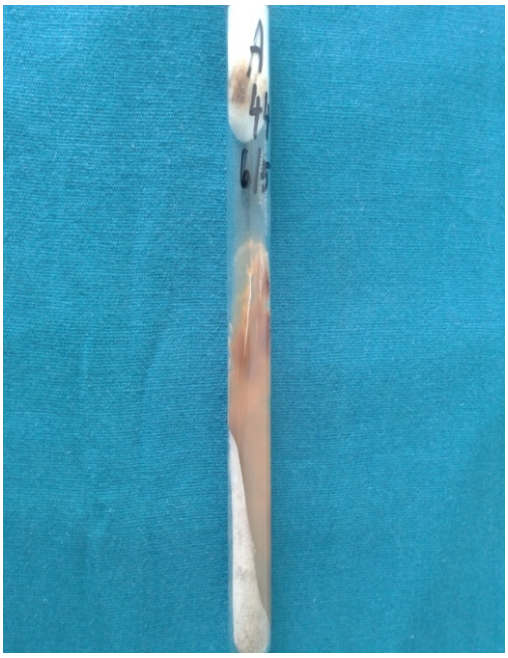


MACROSCOPIC APPEARANCE - *Trichophyton tonsurans*
OBVERSE –White coloured,powdery REVERSE-yellow brown

MICROSCOPIC APPEARANCE
-Varying shapes of microconidia



MACROSCOPIC APPEARANCE - ***Microsporum gypseum***
OBVERSE –Buff coloured,powdery. REVERSE-Orange brown

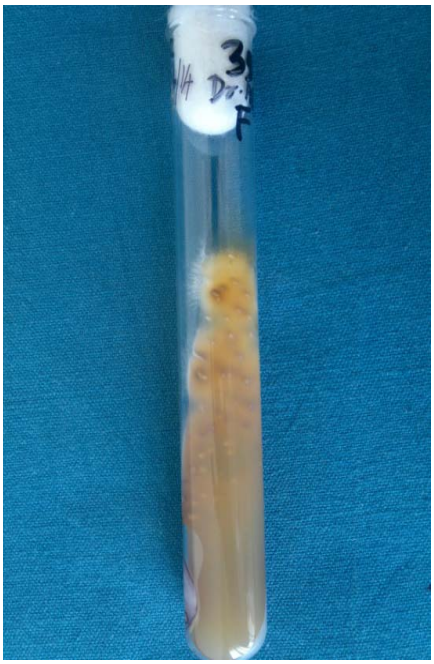


MICROSCOPIC APPEARANCE
Microconidia and macroconidia(2 to 6 celled)

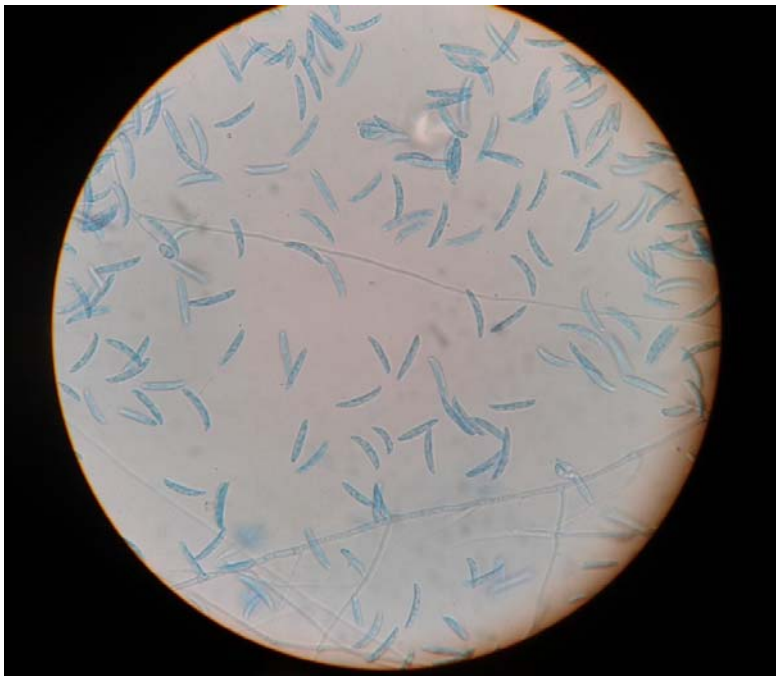
MACROSCOPIC APPEARANCE - *Epidermophyton floccosum*
OBVERSE-Khaki coloured,powdery. REVERSE-yellow-brown

MICROSCOPIC APPEARANCE
Club shaped macroconidia in clusters

MACROSCOPIC APPEARANCE OF *Fusarium* species
OBVERSE (Cottony,Pink coloured) REVERSE

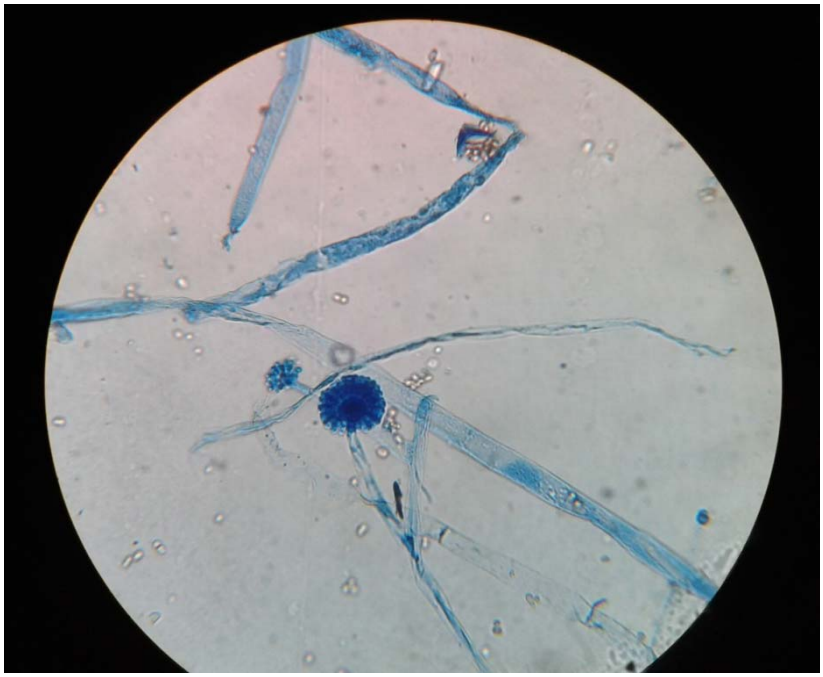


MICROSCOPIC APPEARANCE -Sickle shaped macroconidia



MACROSCOPIC APPEARANCE- *Syncephalustrum* species
OBVERSE –Cottony,gray black. REVERSE

MICROSCOPIC APPEARANCE-globular vesicle supporting merosporangium

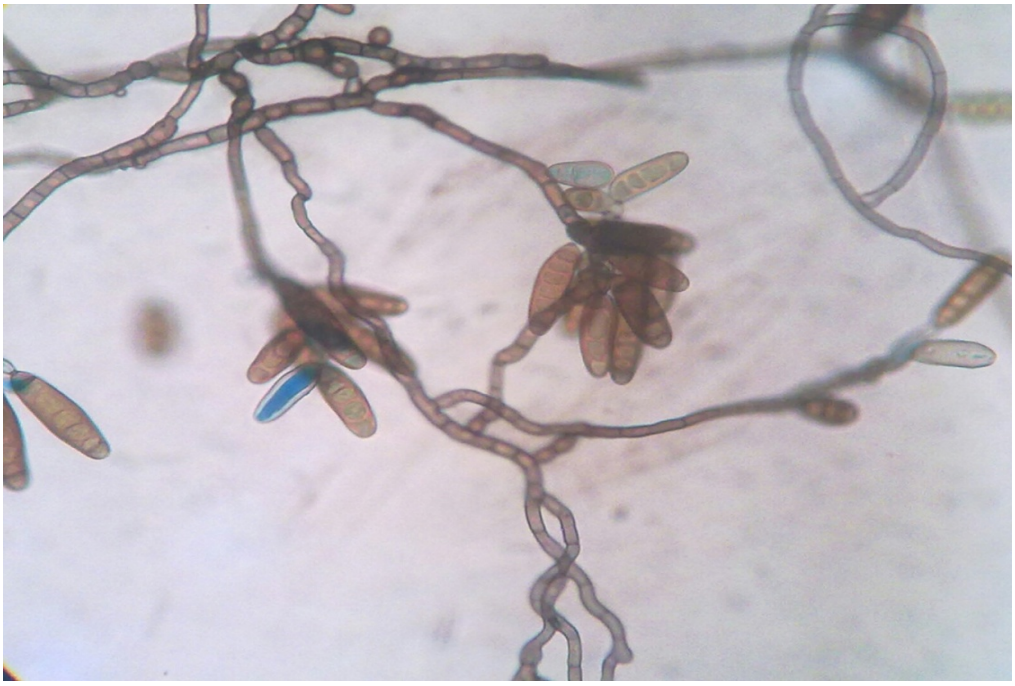


MACROSCOPIC APPEARANCE OF *Bipolaris* species

OBVERSE –Cottony **REVERSE-Black pigmentation**

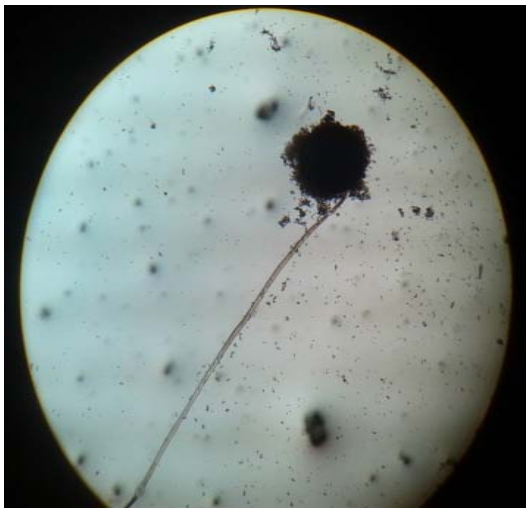
MICROSCOPIC APPEARANCE

Septate dematiaceous hyphae,dark macroconidia

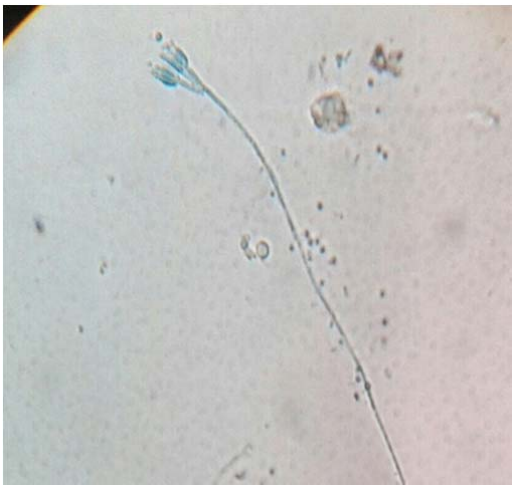


A.fumigatus

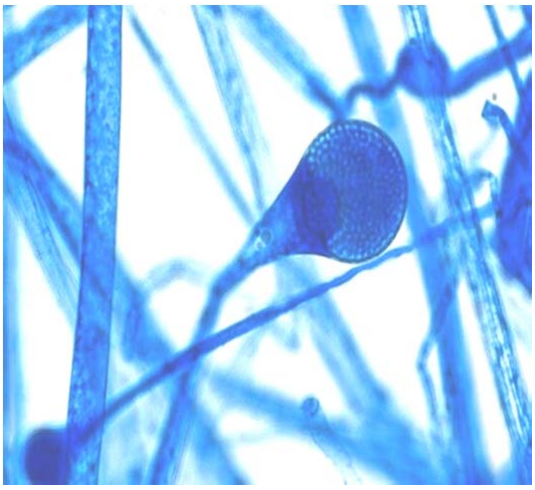
A.niger



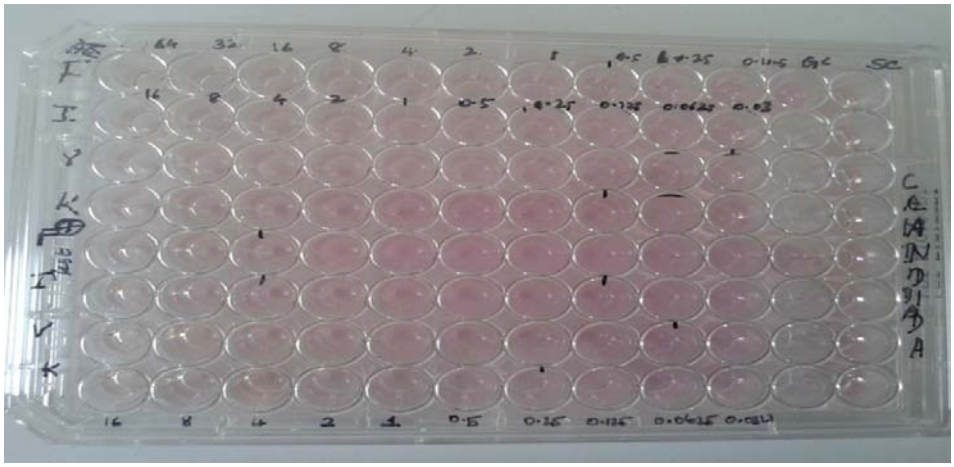
***Penicillium* species**



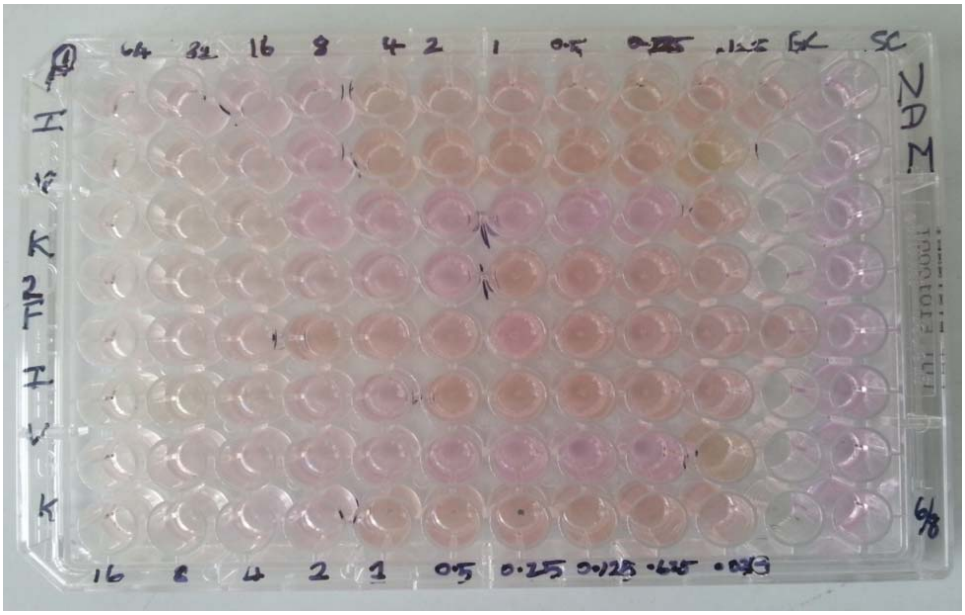
Absidia(Lichtheimia) species



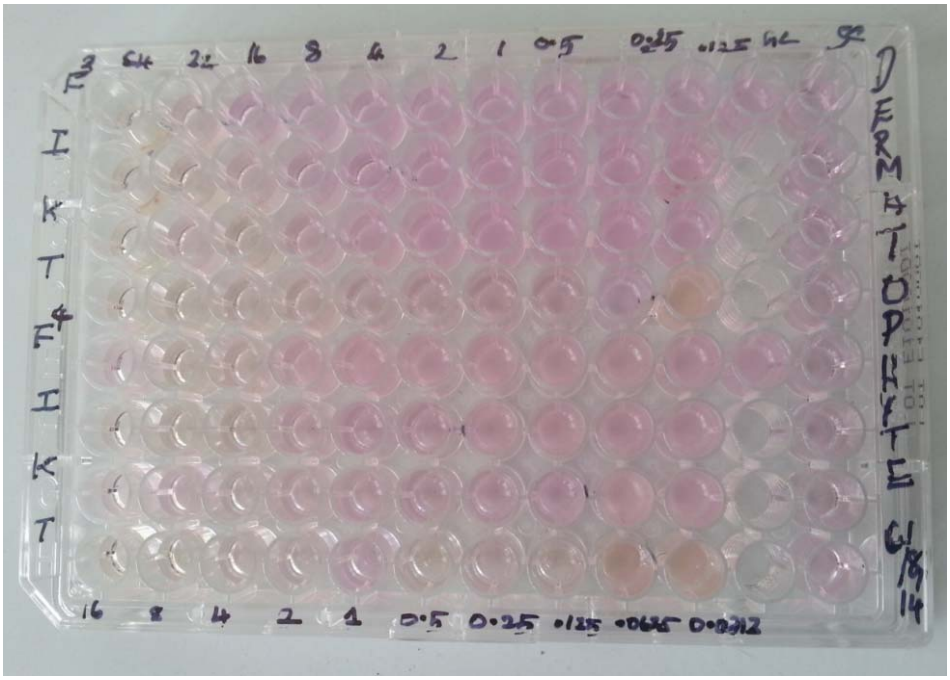
MIC BY BROTH MICRODILUTION METHOD *CANDIDA* SPECIES



MIC DETERMINATION OF NON-DERMATOPHYTIC MOULDS BY MICROBROTH DILUTION METHOD



MIC DETERMINATION OF NON-DERMATOPHYTIC MOULDS BY MICROBROTH DILUTION METHOD



DISCUSSION

DISCUSSION

Onychomycosis is not only a cosmetic problem among diabetics but also a potential health problem among them. Higher frequency of diabetic foot disorders,lower limb amputations are common among diabetic patients than those diabetic patients without onychomycosis[10,98].

A total of 175 diabetic patients who attended the Out patient clinic of the Departments of Diabetology and Dermatology,Rajiv Gandhi Government General Hospital,who were diagnosed with onychomycosis were included in this study for a period of one year.

Both male and females in the age group of 24 to 78 years were included in the study. The majority of the patients(35.43%) were in the age group of 51 – 60 years [TABLE.1] . This is similar to the study by Al-Mutairi *et al* who reported onychomycosis to be the commonest in the age group of 56 to 65 years[98]. Gupta *et al* and Saunte *et al* also have reported a higher prevalence of onychomycosis among the elderly diabetic patients[12][16].

In our study onychomycosis among males (52%) was only marginally higher than females (48%) in the ratio of 1.08:1[TABLE.2]. This can be attributed to the increase in health awareness among women and their positive attitude towards treatment and increased cosmetic consciousness[99]. Higher incidence among males were noted in several studies[98][12][14]. This may be due to the higher outdoor activites among males who were also more prone to trauma[102].

The proportion of onychomycosis among patients in various occupations was analysed. It was observed that 44% of the patients were farmers and 26.28% were housewives. Such increased incidence is due to increased perspiration, greater risk of occupation related trauma and exposure to soil saprophytes. Farmers are more commonly involved due to nail trauma as a result of farming and manual labour which will lead to inoculation and growth of fungi causing onychomycosis[23].

In our study, 92% of study population had Type II diabetes mellitus [TABLE.3]. The most common clinical presentation of onychomycosis was DLSO (89.5%), followed by TDO (9.7%) and PSO (1.14%) [TABLE.4]. This is consistent with the study by Al-Mutairi *et al* [98] and Romano C *et al*[122] who reported the most common presentation as DLSO.They also observed that type II diabetes mellitus patients were more common in their study.

In our study 68% of diabetic patients had toe nail involvement followed by both nails (17%) and finger nails (15%) [TABLE.5]. This is consistent with a study fom India[14] and Kuwait[98]who reported a similar frequency of occurrence in toe nails. Further it was observed that the great toe nail was the most common site probably due to the bigger size predisposing to increased trauma[107].

Conversely, some authors have reported a predominant involvement of finger nails[102]. Increased incidence of finger nail onychomycosis may be due to increased likelihood of occupation related trauma. Finger nail onychomycosis is also more likely than toe nail onychomycosis to arouse the patients' concern, driving them to seek medical attention[102].

In our study, the duration of OM varied between 1 month to more than 5 years. Majority of the patients (52.57%), gave the history of nail involvement for a duration of 1 to 5 years. [TABLE.6]. The mean duration of OM among the study population was 4.4 years. A study by Al-Mutairi *et al*[98] reported that the mean duration of OM was 1.8 years.

In our study, 87% of the patients had mild onychomycosis and 10% of patients had severe onychomycosis [TABLE.7]. The severity of onychomycosis varies in different studies.Severe onychomycosis was reported in 32.5% and 4.4% by Gupta *et al*[16] and Al-Mutairi *et al* [98]respectively.

In our study, there was no statistically significant correlation between the severity of onychomycosis and type of diabetes mellitus (p=0.725)[TABLE.8]. This is well correlated with the study of Saunte DM *et al*[12] and Al-Mutairi *et al* [98].

Majority of the patients had a history of diabetes mellitus for a duration of 1 to 5 years(34.9%) and 5 to 10 years (34.9%). There was no statistically significant correlation between the severity of onychomycosis and the duration of diabetes mellitus(p=0.600)[TABLE.9].This is similar to the observation by Leelavathi *et al* in Malaysia [100] ,Suhelya *et al* [94] and Noorbala[95]. But Al-Mutairi *et al* [98] and Dogra S *et al*[14] reported a siginficant increase in the severity of onychomycosis with longer duration of diabetes mellitus.

There was no statistically significant correlation between the severity of onychomycosis and blood glucose levels of patients with diabetes mellitus in our study (p=0.679) [TABLE.10]. This is also similar to the studies by Al-Mutairi *et al*

[98] , Dogra S *et al*[14] and Glucan A *et al*[105] who found a similar lack of correlation between the two variables.

A study by Romano C *et al*[122] also reported that there was no significant correlation between dermatophytosis and type or duration of diabetes and blood glucose levels.

In our study, the culture positivity rate was 46.28% and the rate of detection in KOH mount was 70.29%[TABLE.11]. A study by Karmakar S *et al* [110] reported an isolation rate of 41.6% .Our isolation rate is also similar to a study by Ahuja *et al*[106] who reported an isolation rate of 39.5% among OM. A lower culture positivity rate of 29% and 20% was observed by Gupta *et al.,* and Malik *et al*[106] respectively.

In our study,2.29% of patients samples were culture positive but potassium hydroxide(KOH) mount negative and 26.29% of cases were KOH mount positive but culture negative[TABLE.12]. A study by Al-Mutairi *et al* [98] reported that 18.6% were culture positive but KOH mount negative and 11.6% potassium hydroxide mount positive but culture negative. This culture negativity inspite of a positive KOH mount ,could be due to the non-viable fungal elements , inadequacy in sampling and non-reported treatment with antifungal agents[110] .As the median time to culture positivity is about 2 weeks ,the efficiency of direct microscopic examination plays an important role in rapid diagnosis and treatment.

In our study, *Candida* species was the most common isolate (39.50%), followed by non-dermatophytic moulds (34.58%) and dermatophytes (25.92%) [TABLE.13]. This is similar to the study conducted by Dogra S *et al* in India[14] and by Glucan *et al*[105].

Conversly Al-Mutairi *et al* [98] in his study on Clinicomycological profile on onychomycosis among diabetic patients reported the predominant pathogen as dermatophytes[98].

The most common *Candida* species isolated was *Candida parapsilosis* (62.5%) followed by *Candida albicans* (25%) and *Candida tropicalis*(12.5%) [TABLE.14]. This is smilar to the study by Gahannaoum *et al*[42], who reported the most common species of *Candida* to be *C.parapsilosis* (66.6%) followed by *C.albicans (16.7%), C.guilliermondii (11.9%), C.tropicalis (2.4%) and C.lusitaniae(2.4%).*This is in contrast to a study by Al-Mutairi *et al* who observed *Candida albicans* as the most common species [98].

The epidemiological pattern of onychomycosis caused by *Candida* species was analysed. It was found that farmers and housewives were most commonly affected. There was a history of trauma in 12.5% of patients[TABLE.15]. Finger nails were the most common site of infection. A study by Rakshit *et al*[117] in North India about *Candida* as an emerging pathogen in onychomycosis,also reported that *Candida* species are associated with finger nail infection among female patients.

Interpretation of isolation of non-dermatophytic moulds from onychomycosis requires caution because of their presence in environment as contaminants. In the present study ,diagnosis was based on English and Walshe criteria. [57][58]. Nondermatophytic moulds were isolated in 34.58% of the study population. This is similar to the study by Leelavathi *et al* [100] who also reported a similar isolation rate of nondermatophytic moulds.

In the present study,among non-dermatophytic moulds(NDM), *Fusarium* species was the most common etiological agent (57.14%), followed by *Aspergillus fumigates* (14.27%), *A.niger* (10.71%), *Bipolaris* species (7.14%) and *Absidia (Lichtheimia)* species, *Penicillium* species and *Syncephalastrum* species (3.58%) each repectively [TABLE.16].

Fusarium species and *Aspergillus niger* were isolated by Al-Mutairi *et al* [98] in 75% and 25% of NDM respectively . There are few reports of isolation of the other agents such as *Bipolaris* species, *Absidia* species, *Penicillium* species and *Syncephalastrum* species among diabetic patients with onychomycosis. But these species were reported in non diabetic patients with onychomycosis by Purnima Barua *et al* [101] and Veer *et al*[102].

The nondermatophytes were isolated in 34.58% of patients whereas dermatophytes were isolated in 25.92% of infection. This may be due to the ubiquity of varied species of fungi in environment as well as the active nature of our lifestyles which increases the vulnerability to trauma. According to Tosti *et al* [104] , the hot and humid subtropical and tropical climates could be the probable cause of increased frequency of nondermatophytic mould infection.

The epidemiological pattern of onychomycosis caused by nondermatophytic moulds was analysed. It was observed that nondermatophytic onychomycosis was predominantly seen in males,elderly and in farmers.Other comorbid conditions was present in 46.43% of patients[TABLE.17]. This is consistent with the study by Hwang SM *et al*[107]. Among the 16 isolates of *Fusarium* species,two of the isolates in our study was isolated in post renal transplant recipients with diabetes mellitus.Lamb *et al* in his study isolated *Fusarium* species in chronic renal failure patients undergoing

hemodialysis.In his study on frequency of onychomycosis in chronic renal failure patients,he stated that diabetics are 88% more likely to develop onychomycosis than non-diabetics[25]. The nondermatophytic moulds depend on the unkeratinized intercellular cement otherwise it will take the advantage of partial denaturing of nail keratin by pre-existing disease or trauma[86].

In our study,among the dermatophytes the most common isolate was *Trichophyton rubrum* (61.90%),followed by*Trichophyton tonsurans* (28.58%), *Epidermophyton floccosum* (4.76%) and *Microsporum gypseum* (4.76%) [TABLE.18]. *T.rubrum* was the most common etiological agent among dermatophytes which correlates well with other studies[12][98] which have reported higher occurrence of *T.rubrum* infection among diabetic patients with onychomycosis. Al-Mutairi *et al* observed a similar occurrence of dermatophytic infection contributed by pathogens such as *T.rubrum*(72%), *T.tonsurans*(5.5%), *M.gypseum*(5.5%) and *T.mentagrophytes* (16%)[98].

Conversely, Romana C *et al*[122] reported the predominant pathogen as *T.mentagrophytes* in his study on prevalance of dermatophytic skin and nail infection among diabetics.

Extraungual site involvement was present in 52.38% of dermatophytic nail infections.Tinea corporis was found to be associated with 38.46% of *T.rubrum infection*. Tinea pedis was found to be associated with 33.33% of *T.tonsurans*. *Epidermophyton floccosum* was found to be associated with both tinea corporis and tinea cruris.[TABLE.19]. Szepietowski *et al* [36] observed the coexistense of toe nail onychomycosis with tinea pedis (33.8%) , tinea cruris (4.2%), tinea corporis(2.1%),tinea capitis(0.5%),tinae manuum (1.6%) and hypothesized that

infected toenails may be a site from which the mycotic infections can spread to other body areas.Therefore,in diabetic patients effective therapy for onychomycosis is essential not only to treat the affected toenails but also to prevent spreading the infection to other sites .

The choice of antifungal agent is different in yeasts,filamentous moulds and dermatophytes. The drug of choice of OM in diabetic patients is same as that of the patients without diabetes. But elderly diabetic patients require a longer duration to treat and there may be associated complications like cellulitis, diabetic foot ulcers and gangrene, which will increase the admission rates in hospital and surgical interventions[89]. Onychomycosis may also serve as a reservoir of infection. Reports of drug-resistant *Candida* species in onychomycosis have not appeared, although antifungal resistance to fluconazole has been documented in oral candidiasis in immunocompromised patients[35]. By doing *in-vitro* antifungal susceptibility an effective antifungal agent can be selected for treatment and thus helps in optimizing the therapy[69].

All the three species of *Candida* namely *C.parapsilosis,C.albicans* and *C.tropicalis* were sensitive to fluconazole and voriconazole by disk diffusion method[TABLE.20]. The MIC of these isolates to itraconazole and voriconazole were in the range of 0.0625-0.125 µg/ml and 0.0313µg/ml respectively[TABLE.21].All of the isolated *Candida* species were sensitive to itraconazole and voriconazole and found to have lower MIC ranges [108].This was in consistent with a study by Bueno *et al* [119] who observed that itraconazole and voriconazole were the most active agents.

The MIC range of the three *Candida* species for ketoconazole was 0.5-1 µg/ml . A study by Ozcelik *et al* [109]showed that all *Candida* isolates were in the MIC range of about 0.03-32 µg/ml for ketoconazole .Some of the *C.albicans*(n=14) isolates showed intermediate susceptibility (16-32µg/ml) in his study.

The MIC range of *Candida parapsilosis,Candida albicans* and *C.tropicalis* for fluconazole were 1- 2 µg/ml.All of the isolates were found to be sensitive. A study by Espinel Ingroff reported the MIC range of *Candida parapsilosis,Candida albicans* and *C.tropicalis* for fluconazole as 0.25- 8,0.12 – 64 and 0.25 – 64 µg/ml respectively[115].

All of the *Candida* species were sensitive to voriconazole and fluconazole in both disk diffusion and microbroth dilution method. Results of disk diffusion correlated well with the MIC by microbroth dilution method. Therefore, disk diffusion test can be used as an cost effective alternative to microbroth dilution technique. This has also been suggested by a study by Deepak kumar *et al* [111].

The MIC range of *Fusarium* species for voriconazole in our study was 4- 8 µg/ml[TABLE.22]. This is similar to a study by Espinel Ingroff[115] who reported a MIC range of 4 to 16 µg/ml. Other studies have reported a lower MIC range of 0.5 to 4 µg/ml [112,116] .

The MIC range of *Fusarium* species for itraconazole in our study was 8-16 µg/ml. A study by Espinel Ingroff [115] for *Fusarium* species, the MIC range of itraconazole was reported to be 1-16 µg/ml.

The MIC range of *Fusarium* species for ketoconazole in our study was 16 - 32 µg/ml which is similar to the MIC range of Pujol *et al*[113] (1.60–51.20 µg/ml) and by Sekhon AS *et al*[114] who reported the range as 3.125-100 µg/ml.

The MIC range of *Fusarium* species for fluconazole in our study was 32- 64 μg/ml which is similar to that of the study by Bueno *et al*[119] who reported a MIC range of 4-64 μg/ml.

In our study, the MIC values for all of the evaluated drugs with *Fusarium* species were found to be higher than those obtained against the other NDM, which is in agreement with the poor therapeutic response to fungal infections caused by *Fusarium* species as reported in some studies[119,120,121].

The MIC range of *Aspergillus* species in our study for voriconazole was 0.0313 μg/ml which correlates with study by Espinel Ingroff (0.0625-1 μg/ml)[115] . The MIC range of itraconazole in our study was 0.5-1 μg/ml . A higher MIC range of itraconazole was observed by Espinel Ingroff (0.12 – 16 μg/ml) for *Aspergillus* species.

The MIC range of *Bipolaris* species for voriconazole and itraconazole were 0.0625μg/ml and 1 μg/ml respectively which was similar to the observation of Espinel Ingroff (Vori-0.12–1.0 μg/ml and Itra-0.03-0.12 μg/ml**)** [115].

The MIC range of voriconazole was found to be lower when compared to other azole drugs for all non-dermatophytic moulds and can be used as an alternative drug in immunosuppressed patients.

The MIC range of dermatophytes in our study was, for Fluconazole:4-32 μg/ml, Ketoconazole : 0.5 – 2 μg/ml, Itraconazole : 0.25-1 μg/ml and Terbinafine: 0.0313μg/ml [TABLE.23] .Terbinafine was found to have the lower MIC ranges among the evaluated drugs.

Our study correlates with the study by Pankajalakshmi[118] who observed the MIC range of Ketoconazole,Itraconazole and Terbinafine as 0.1 – 10µg/ml ,0.01-1µg/ml and 0.001-0.01 µg/ml respectively. A study by M.A.Ghannoum *et al* [72] reported the MIC range of Fluconazole,Ketoconazole,Itraconazole and terbinafine as 0.12-16 µg/ml , 0.12-64 µg/ml, 0.001-0.05 µg/ml and 0.001-0.5 µg/ml respectively.In his study the MIC of ketoconazole was found to be higher. This variability may be due to important methodological differences among laboratories like incubation temperature.[70]

However, for onychomycosis,MIC breakpoints for diabetes mellitus patients have not yet been established,it remains unclear whether the *in vitro* activity of antifungal drugs is predictive of the clinical outcome[119].

SUMMARY

SUMMARY

About 175 clinically diagnosed cases of onychomycosis in diabetic patients attending the outpatient clinic of Dermatology and Diabetology departments, Madras Medical college & RGGGH for a period of one year were included in the study. Nail scrapings and sub ungual debris were collected from the patients and subjected to direct examination and culture.

- Majority of the patients were in the age group between 51-60 years(35.43%).
- The male to female ratio was 1.08 : 1.
- Farmers were most commonly involved (44%) followed by housewives (26.28%).
- Majority of the study population had Type II diabetes mellitus (92%) .
- Most of the patient had nail affiliction(52.57%) for a duration of 1 to 5 years.
- Toe nail onychomycosis was found in 68% of patients followed by both nail involvement(17%) and finger nails in 15% of patients.
- The most common clinical presentation was DLSO(89.14%) .
- The commonest degree of nail involvement was mild type of onychomycosis.(87%) .
- There was no statistical significance between severity of onychomycosis and type of diabetes mellitus, blood sugar levels and duration of diabetes mellitus .

- The culture positivity rate was 46.28% and potassium hydroxide mount was positive in 70.29% of patients.

- *Candida* species was the most common etiological agent(39.50%) followed by non-dermatophytic moulds (34.58%)and dermatophytes(25.92%).

- Among the *Candida* species ,*C.parapsilosis* was the most common etiological agent(62.5%).*C.albicans* (25%) and *C.tropicalis* (12.5%) were the other pathogens.

- Among the patients with Candidial Onychomycosis , female patients(65.62%) ,farmers (37.5%)and housewives(31.25%) were involved commonly. Finger nails(43.75%) were found to be involved most commonly.

- Among non-dermatophytic moulds,*Fusarium* species was the most common etiological agent (57.14%) followed by *A. fumigatus* (14.27%), *A.niger* (10.71%),*Bipolaris* species(7.14%), *Absidia* species (3.58%), *Penicillium* species (3.58%) and *Syncephalastrum* species (3.58%) .

- Among non-dermatophytic onychomycosis, elderly males(60.72%), farmers (50%) and patients with a H/O.trauma (21.43%) were involved predominantly. Toe nails(75%) were found to be involved commonly.

- Among the dermatophytes, *T.rubrum* was the predominant agent isolated (61.90%) followed by *T.tonsurans* (28.58%) and *E.floccosum*(4.76%) , *M.gypseum* (4.76%) .

- Extaungual site involvement was present in 52.38% of dermatophytic nail infection.

- Tinea corporis was found to be associated with 38.46% of *T.rubrum infection*. Tinea pedis was found to be associated with 33.33% of *T.tonsurans*. *Epidermophyton floccosum* was found to be associated with both tinea corporis and tinea cruris.

- Antifungal susceptibility testing was done by disk diffusion method and microbroth dilution method for *Candida* species.

- All the *Candida* species were sensitive to azole drugs .

- There was a good correlation between disk diffusion test and microbroth dilution method for *Candida* species.

- For nondermatophytic moulds except *Fusarium spp* ,voriconazole was found to have a lower MIC range than other azole drugs .

- *Fusarium* species had a higher MIC range for azole drugs.

- Terbinafine was found to have lower MIC range in case of dermatophytes.

- Itraconazole was found to have a lower MIC range among the evaluated azole drugs in dermatophytes.

CONCLUSION

CONCLUSION

A clinical suspicion of onychomycosis is crucial in patients with diabetes mellitus.Type II diabetes mellitus patients were involved most commonly and the most common clinical presentation among them was distal lateral subungual onychomycosis.Majority of the patients had mild onychomycosis.A siginificant group of patients had extraungual site involvement.

Our study revelaed that there was no correlation between severity of onychomycosis and type of diabetes mellitus,duration of diabetes and blood sugar levels.

A combination of direct examination and culture would yield the best results for identification of the exact pathogen and for confirmation of diagnosis.

This study highlights the role of *Candida parapsilosis* as a primary pathogen in diabetic patients with onychomycosis. Fluconazole still remains as an effective antifungal agent among the *Candida* species.

Non dermatophytic moulds ,which are generally considered as contaminants , are now emerging as primary pathogens, though their definitive diagnosis should be done with caution.Repeated isolation on consecutive sampling will help to ensure the clinical significance of the isolates.*Fusarium* species which can cause disseminated infections in immunocompromised patients were the predominant nondermatophytic pathogens.

Dermatophytes were less commonly isolated than *Candida* species and nondermatophytic moulds,*T.rubrum* being the most common isolate. Terbinafine was found to have a lower MIC range against dermatophytes.

The *in vitro* antifungal susceptibility patterns showed a comparable results with previous studies.However,for onychomycosis, clinical breakpoints have not yet been estabilished .Therefore it remains unclear whether the *in vitro* activity of antifungal drugs is predictive of clinical outcome. Hence, routine antifungal susceptibility testing along with the clinical outcome should be performed in future studies.

In addition, the diabetic patients should be educated regarding the importance of foot and nail care and early treatment should be considered as an essential component in the management of these patients.

APPENDICES

APPENDIX - 1

LIST OF ABBREVIATIONS USED

ATCC	-	American type culture collection
CO	-	Candidial onychomycosis
CLSI	-	Clinical Laboratory Standard Institute
DM	-	Diabetes Mellitus
DMSO	-	Dimethyl Sulfoxide
DLSO	-	Distal and lateral subungual onychomycosis
TM	-	Dermatophyte Test Medium
EO	-	Endonyx Onychomycosis
HIV	-	Human Immunodeficiency Virus
HPE	-	Histopathological Examination
KOH	-	Potassium hydroxide
LPC	-	Lactophenol Cotton Blue mount
MO	-	Mixed Onychomycosis
MIC	-	Minimum Inhibitory Concenteration
NDM	-	Non Dermatophytic Moulds
NDO	-	Nondermatophytic Onychomycosis
OM	-	Onychomycosis
PAS	-	Periodic Acid Schiff
PDA	-	Potato Dextrose Agar
PCR	-	Polymerase Chain Reaction
PSO	-	Proximal subungual onychomycosis
RPMI	-	RoseWell Park Memorial Institute
SDA	-	Sabourauds Dextrose Agar
SO	-	Secondary Onychomycosis
SWO	-	Superficial White Onychomycosis
TDO	-	Total Dystrophic Onychomycosis
YNB	-	Yeast Nitrogen Base

APPENDIX – 2

MEDIA ,STAINS AND REAGENT

Sabouraud Dextrose Agar with Antibiotics

Composition of Sabouraud Dextrose Agar

Dextrose : 20 gm

Peptone : 10 gm

Agar : 20 gm

Distilled Water : 1000 ml

Final pH : 6.9

The ingredients are dissolved by boiling. Chloramphenicol(50mg/lit) and Cycloheximide(500mg/lit) was added . Chloramphenicol was dissolved in 10 ml of 95% ethanol and added to boiling medium. Cycloheximide was dissolved in 10 ml of acetone and added to the boiling medium. Autoclave at 121°C for 15 minutes, Dispense in sterile tubes and allow to cool in slanted position.

DERMATOPHYTE TEST MEDIUM:

Papaic digest of Soyabean : 10gm

Glucose :10gm

Phenolred :0.20gm

Agar : 20gm

Actidione :500mg

Gentamicin :100mg

Final pH: 5.5 20.1gm of powder is dissolved in 500ml of distilled water. Heat to boiling to dissolve the medium completely.Autoclaved at 121°C for 15 mins and dispensed in test tubes.

POTATO DEXTROSE AGAR

Potato Infusion : 200 g

Dextrose : 20 g

Agar : 15 g

Distilled Water : 1 Lit

Final pH: 5.6

39 grams of media is suspended in 1000 ml of distilled water.Heat to boiling to dissolve the medium completely. Autoclave at 121^0 C for 15 minutes. Cool to 50^0 C & pour into sterile test tubes/petridish.

CORN MEAL AGAR MEDIUM:

Cornmeal : 8gm

Agar : 4gm

Tween 80(1%): 2ml

Distilled water : 200ml

Heat cornmeal and water at 60ºC for 1 hour and filter through filter paper.Add distilled water to make it 200ml and then add agar.Tween 80 is then added.Autoclave it at 121 ºC for 15 mins.

YEAST NITROGEN BASE MEDIUM

Part A : Agar 40gms/lit

Part B : Yeast nitrogen base 6.7gms/lit

40 grams of part A media is suspended in 900 ml of distilled water. Heat to boiling to dissolve the medium completely.Autoclave at 121º C for 12 minutes.Cool to 50° C and mixed with sterile part B solution aseptically.

CHROMAGAR CANDIDA MEDIUM:

Ingredients	Gms/L
Peptone	15.00
Yeast extract	4.00
Dipotassium hydrogen phosphate	1.00
Chromogenic mixture	7.22
Chloramphenicol	0.50
Agar	15.00

Final pH: 6.3.

42.72 grams of media is suspended in 1000 ml of distilled water.Heat to boiling to dissolve the medium completely. Donot autoclave. Cool to 50° C and pour in sterile petridish.

MUELLER HINTON AGAR :

Ingredients	**Gms / Litre**
Beef extract	3.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25°C) 7.3±0.2

38grams of media is suspended in 1000 ml of distilled water. Add 20gm of glucose(2%) and methylene blue (0.5µg/ml) is added. Dissolve the medium completely. Dispense and sterilize by autoclaving at 115-121°C for 10 minutes. DO NOT OVERHEAT.

CHRISTENSEN'S UREASE TEST MEDIUM:

Peptone	:1g
Sodium chloride	:5g
Dipotassium hydrogen phosphate	:2g
Phenol red	:6ml
Agar	: 20g
Distilled water	:1 ltr

10% sterile solution of glucose l0ml

Sterile 20% urea solution l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and the tube is placed as slopes.

SUGAR FERMENTATION TEST MEDIUM:

To the basal medium of peptone water, add sterilised sugars of 2%.

Indicator : Bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar 2ml

Dislilled water l00ml.

RPMI 1640 MEDIUM[With glutamine and without bicarbonate]

Obtained commercialy as a dehydrated powder.Suspend 8.4gms of media in 900ml of sterile distilled water.Stir it to completely dissolve the medium donot heat.Sterilize the medium by filtration.Final pH=7.0.

GRAM STAINING

Methyl violet (2%) : l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)

Grams Iodine : l0g Iodine in 20g KI (fixative)

Acetone : Decolourising agent

Carbol fuchsin : 1% Secondary stain

POTASSIUM HYDROXIDE MOUNTS

It is prepared from the following ingredients

Potassium hydroxide : 20 gms

Glycerol : 10 ml

Distilled water : 80 ml

To a solution of 20% KOH, 10% Glycerol is added to prevent drying.Mix ingredients and store at room temperature. For 40% KOH 40gms of potassium hydroxide is added.

LACTOPHENOL COTTON BLUE STAIN

The lacto phenol cotton blue (LPCB) is used to study the morphological features of the fungal isolates.

It contains the following ingredients:

Melted phenol : 20 ml

Lactic acid : 20 ml

Glycerol : 40 ml

Cotton blue : 0.05 gm

Distilled water : 20 ml

Mix all the reagents properly and dissolve 0.05 g of cotton blue stain in the distilled water before mixing with the remaining reagents. The phenol acts as disinfectant, lactic acid preserves the morphology of the fungi and glycerol is hygroscopic agent which prevents drying. The cotton blue stains the outer wall of the fungus. Tease out of a fragment of the culture on a glass slide in a drop of LCB using two teasing needles. Put of a coverslip and examine under the microscope. If the plane LCB is used the edges of the coverslip can be sealed with nail polish to keep it for longer period of time.

ANNEXURES

ANNEXURE – 1

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. A.Gomathi Chitra,
Post Graduate in MD Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai-3.

Dear **Dr. A.Gomathi Chitra,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled ***" A Study on Mycological profile of Onychomycosis in Diabetic patients And Their Anti Fungal Susceptibility Pattern"*** No.14122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

13/12/13

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE – 2

PROFORMA

Name : OP NO:

Age: Ward:

Sex:

Occupation:

Address:

Presenting complaints

Personal history

Past history

Prior antifungal therapy

Microbiological investigation:

Direct examination - KOH:

Culture : Fungal culture

Speciation

For *Candida* species

CHROM agar medium
Cornmeal agar medium
Urease test
Germ tube test
Assimilation and Fermentation of sugars

For *Trichophyton* species

Urease test
Lactophenol cotton blue mount
The Microslide culture

Antifungal sensitivity pattern:-

ANNEXURE - 3

INFORMED CONSENT FORM

STUDY TITLE : "A study on Mycological profile of Onychomycosis in Diabetic patients in a Tertiary care Hospital and their antifungal susceptibility pattern"

I……, hereby give consent to participate in the study conducted by Dr.A. Gomathi Chitra, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (nail scrapings/subungual debris) for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Place

Of the patient/ relative Date

Patient Name & Address:

Signature of the investigator:

Signature of guide:

MASTER CHART

S NO	NAME	AGE	SEX	OCCUPATION	IP/OP	ONYCHOMYCOSIS TYPE	ONYCHOMYCOSIS_DURATION years	DURATION_DM YEARS	DM TYPE	BLOOD SUGAR in mg/dl	NO.of nails	FN/TN/Both	KOH	ASSOCIATED FACTORS	FINDIGS	MIC FLU	MIC KET	MIC ITRA	MIC VORI	MIC TERBI
1	Mumtaj	42	F	Housewive	197202	DLSO	6months	4	2	194	2	TN	P		*C.parapsilosis*	2	1	0.125	0.0313	
2	Patamma	70	F	Farmer	39502	DLSO	1	2	2	200	2	TN	P		*C.parapsilosis*	1	0.5	0.0625	0.0313	
3	Yasodha	34	F	Farmer	8667	DLSO	4 months	2 1/2months	1	135	1	TN	P		*NG*					
4	Lilly	65	F	Housewive	278501/96	DLSO	3	10	2	130	1	TN	P	HT	*Syncephalastrum species*	4	0.5	0.0625	0.0313	
5	Kothandam	71	M	Farmer	2202/13	DLSO	6months	6	2	180	1	TN	P		*NG*					
6	Rajasekar	66	M	Driver	11109	DLSO	4	4	2	142	1	FN	P	HT	*C.tropicalis*	2	0.5	0.0625	0.0313	
7	Kumar	59	M	Tailor	68754/11	DLSO	5	5	2	166	1	TN	P		*NG*					
8	Akthar Basha	60	M	Watch man	120774	TDO	5months	2	2	170	1	TN	P		*C.albicans*	2	1	0.125	0.0313	
9	Kasthuri	54	F	Housewive	3455	DLSO	3	6	2	266	1	TN	P		*Fusarium species*	64	32	16	4	
10	Indhrani	65	F	Housewive	9643	DLSO	5	15	2	244	1	TN	N		*NG*					
11	Lakshmanan	65	M	Driver	68112/08	DLSO	4	10	2	208	1	TN	N		*NG*					
12	Pandiyan	55	M	Farmer	6599/13	DLSO	1	2	2	146	3	Both	N		*NG*					
13	Maliga	50	F	Housewive	11180	DLSO	4	1month	2	310	2	Both	P		*Fusarium species*	64	32	16	4	
14	Devi	45	F	Housewive	36/13	DLSO	2	7	2	197	1	TN	P		*NG*					
15	Rabbakal	52	F	Housewive	9201	DLSO	3	6months	2	189	2	TN	N		*NG*					
16	Ellammal	58	F	Housewive	207784	DLSO	5	18	2	160	10	TN	P		*C.parapsilosis*	2	1	0.125	0.0313	
17	Doss	62	M	Farmer	512/13	DLSO	5	10	2	140	2	TN	P		*NG*					
18	Raja	39	M	Engineer	178521	DLSO	6months	4	2	164	2	TN	N		*NG*					
19	Vasanthi	58	F	Staff nurse	178523	DLSO	8months	3	2	198	1	FN	N		*NG*					
20	Suryakumari	52	F	Housewive	15821	DLSO	5months	3	2	110	2	TN	P	H/Otrauma	*C.parapsilosis*	2	0.5	0.0625	0.0313	
21	Sasikumar	24	M	Engineer	11492	DLSO	2months	newly detected	1	259	1	FN	P		*NG*					
22	Thilagarasan	43	M	Clerical staff	77672	DLSO	1month	6	2	110	7	Both	N		*NG*					
23	Ellumalai	35	M	Carpenter	22213	DLSO	3months	2	1	190	1	TN	P		*Fusarium species*	64	32	8	4	
24	Dharma	75	M	Farmer	2421	DLSO	4months	6months	2	150	1	Both	N		*NG*					
25	Pechiyammal	65	F	Farmer	11239	DLSO	8	10	2	210	1	Both	P	HT	*C.parapsilosis*	2	1	0.0625	0.0313	
26	Jalandar	55	M	Watch man	31464	DLSO	1	3	2	180	2	TN	P	HT	*C.parapsilosis*	2	0.5	0.125	0.0313	
27	Velu	55	M	Watch man	38401	DLSO	2	8	2	300	2	TN	N		*NG*					
28	Pachaiammal	47	F	Housewive	15904	DLSO	6	2	2	125	2	TN	N		*NG*					

29	Ramesh	43	M	Driver	43003	DLSO	1month	2	1	160	1	TN	P		*Penicillium Species*	1	8	1	0.0313	
30	Shobana	40	F	Clerical staff	5485	DLSO	1	3months	1	220	1	TN	P		*Fusarium species*	64	32	16	4	
31	Udhayakumar	50	M	Carpenter	25387	DLSO	6months	3months	2	172	1	TN	P		*NG*					
32	Dhamodharan	62	M	Farmer	35246	TDO	6	8	2	200	20	Both	P		*Microsporum gypseum*	8	2	1		0.031
33	Jagadeswari	58	F	Housewive	35346	DLSO	4	6	2	140	1	TN	N		*NG*					
34	Rasathi	46	F	Clerical staff	1210/14	DLSO	1	4	2	182	10	Both	P	H/o frequent hand washing	*C.albicans*	1	1	0.0625	0.0313	
35	Lakshmanan	36	M	Sweeper	23474	DLSO	2	1	2	190	2	TN	N		*NG*					
36	Rajendran	55	M	Farmer	1364/14	DLSO	5	7	2	152	2	Both	P	Tineae cruris&T.capitis	*Trichophyton tonsurans*	8	2	0.5		0.031
37	Raju	64	M	Carpenter	1192/14	DLSO	1	1	2	160	1	TN	P		*NG*					
38	Selvam	47	M	Farmer	1452/14	DLSO	1	6months	2	210	1	TN	P		*C.parapsilosis*	1	0.5	0.0625	0.0313	
39	Nagarajan	75	M	Carpenter	22542	DLSO	5	10	2	140	2	TN	N		*NG*					
40	Kamalakshi	56	F	Tailor	29230	DLSO	1month	12	2	145	1	TN	P	HT	*C.parapsilosis*	2	1	0.125	0.0313	
41	Seshan	48	M	Driver	45693	TDO	15	1	1	110	20	Both	P		*NG*					
42	Gajendran	50	M	Farmer	56412	DLSO	1month	4	2	120	1	TN	N	T.corporis	*NG*					
43	Shanmugam	45	M	Shopkeeper	21424	DLSO	2	4	2	125	7	Both	N		*NG*					
44	Datchayani	46	F	Farmer	6671/13	DLSO	10	5	2	300	1	TN	P	T.corporis & T.glutealis	*Trichophyton rubrum*	32	0.5	0.5		0.031
45	Amrunisha	54	F	Farmer	1544/14	DLSO	5months	6	2	210	13	Both	P		*Trichophyton rubrum*	8	2	0.25		0.031
46	Murugan	50	M	Farmer	2193	TDO	3	6months	2	174	2	TN	P		*Trichophyton rubrum*	32	1	0.25		0.031
47	Abdullah	54	M	Shopkeeper	57077	PSO	6months	8	2	180	11	Both	N		*NG*					
48	Sowdhamani	57	F	Housewive	1576/14	DLSO	1	7	2	132	1	TN	N		*Trichophyton rubrum*	16	2	0.25		0.031
49	Kasi	64	M	Farmer	49598	DLSO	4	5	2	126	11	Both	N	HT	*NG*					
50	Mary	40	F	Sweeper	52712	DLSO	5	4	1	142	1	FN	P		*Trichophyton tonsurans*	4	1	0.5		0.031
51	Ravichandran	49	M	Farmer	25421	DLSO	5months	2months	2	355	2	TN	P		*Absidia*	16	8	4	1	
52	Valmiki	70	M	Farmer	48521	DLSO	1	6	2	192	2	TN	N	,Trauma	*NG*					
53	Bakyam	53	F	Housewive	2641	DLSO	2	18	2	160	4	Both	N	T.corporis	*NG*					
54	Chandru	34	M	Engineer	57303	DLSO	3months	5months	2	180	2	TN	P		*NG*					
55	Kaniyappan	38	M	Farmer	67412	DLSO	2	2	2	178	3	TN	P	CKD,HT,H/O Trauma	*Fusarium species*	64	16	16	4	
56	Arulmary	55	F	Staff nurse	17128	DLSO	2	4	2	380	1	TN	N	H/O trauma	*NG*					

57	Ramaayi	58	F	Housewive	18221	DLSO	3	4	2	160	2	TN	N	T.corporis,Hypotyroidism	*Trichophyton rubrum*	32	1	0.25		0.031
58	Anbupriya	44	F	Farmer	51422	DLSO	4	2	2	125	7	TN	N		*Trichophyton tonsurans*	4	1	0.5		0.031
59	Periyanna	48	M	Farmer	14821	DLSO	2	8	2	120	1	TN	N		*NG*					
60	Nagoreamma	55	F	Farmer	61121	DLSO	5	2	2	180	2	TN	P	H/O trauma	*Fusarium species*	64	32	16	4	
61	Rajalakshmi	60	F	Farmer	55770	TDO	10	12	2	130	2	Both	P		*C.parapsilosis*	1	1	0.0625	0.0313	
62	Perumal	62	M	Farmer	51720	DLSO	7	10	2	128	3	Both	P		*A.fumigatus*	16	2	0.5	0.0313	
63	Thangaraj	52	M	Farmer	52141	DLSO	4	6	2	140	2	TN	P		*Trichophyton rubrum*	8	0.5	5		0.031
64	Thangam	54	F	Housewive	12814	DLSO	6	6	2	128	6	Both	N		*NG*					
65	Anbu	49	M	Carpenter	621412	DLSO	5	7	2	130	1	TN	P		*NG*					
66	Deepa	38	F	Engineer	52770	DLSO	2	3	1	128	1	TN	P		*NG*					
67	Raju	72	M	Farmer	61012	DLSO	5	18	2	130	2	TN	P		*NG*					
68	Sivukur	56	M	Farmer	62421	DLSO	6	7	2	160	1	FN	P		*C.albicans*	2	0.5	0.0625	0.0313	
69	Rakamma	62	F	Housewive	614212	DLSO	4	12	2	142	2	TN	P		*NG*					
70	Agilambal	70	F	Housewive	62141	DLSO	5	15	2	136	3	TN	N		*NG*					
71	Govindhammal	50	F	Staff nurse	65012	DLSO	4	4	2	140	2	TN	P	T.corporis	*Trichophyton rubrum*	8	0.5	0.5		0.031
72	Madan	51	M	Farmer	31584	DLSO	6months	4	2	142	1	FN	P		*Trichophyton rubrum*	8	0.5	0.5		0.031
73	Parthiban	39	M	Farmer	74234	DLSO	5months	1	2	170	2	TN	P		*Fusarium species*	64	32	8	4	
74	Ambika	47	F	Housewive	54634	DLSO	6	1	2	160	2	TN	N	H/O trauma	*NG*					
75	Baskaran	48	M	Tailor	93667	DLSO	11/2 months	6months	2	152	2	TN	P	H/O trauma	*Bipolaris Species*	8	4	1	0.0625	
76	Gracy	58	F	Housewive	82039	TDO	1	8	2	130	20	Both	P		*Fusarium species*	32	32	8	4	
77	Malathy	45	F	Farmer	47234	DLSO	2	5	2	152	2	FN	P		*C.parapsilosis*	2	0.5	0.0625	0.0313	
78	Galil	49	M	Shopkeeper	156241	DLSO	4	8	2	126	2	Both	N		*NG*					
79	Pakkirrmaideen	55	M	Watch man	72019	DLSO	4	1	2	180	2	TN	N		*NG*					
80	Nabeesha Begam	55	F	Housewive	45234	DLSO	2	4	2	119	11	Both	P		*NG*					
81	Aadi moolam	45	M	Tailor	42234	TDO	2	1	2	200	1	TN	P	H/O trauma	*A.fumigatus*	16	2	0.5	0.0313	
82	Malar	45	F	Farmer	90562	TDO	8	6	2	146	20	Both	P		*NG*					
83	Kasi	64	M	Farmer	9422	DLSO	3	4	2	132	1	TN	P		*Trichophyton rubrum*	32	0.5	0.5		0.031
84	Abdullah	68	M	Shopkeeper	104747	DLSO	4	10	2	162	1	TN	P		*NG*					

85	Fatima	42	F	Housewive	18896	DLSO	3	6	2	142	1	TN	P	T.pedis	*Trichophyton tonsurans*	8	0.5	0.25		0.031
86	Vinitha	38	F	Engineer	125802	DLSO	4	3	2	160	2	TN	N		*NG*					
87	Venugopal	64	M	Tailor	55861	DLSO	1month	8years	2	197	1	TN	P	HT	*A.niger*	8	1	0.5	0.0313	
88	Devarajan	69	M	Carpenter	105398	DLSO	4	11	2	146	1	FN	P		*A.niger*	4	2	1	0.0313	
89	Kulanthaisamy	60	M	Farmer	61824	DLSO	5	8	2	142	1	FN	N		*NG*					
90	Bhuvaneswari	60	F	Housewive	114650	DLSO	2months	6	2	140	1	TN	P	T..corporis	*Trichophyton rubrum*	32	0.5	0.5		0.031
91	Sameul	39	M	Tailor	302083	DLSO	3months	4	2	126	1	TN	P		*Bipolaris Species*	8	2	1	0.0625	
92	mahalakshmi	58	F	Housewive	62155	DLSO	4	8	2	148	2	Both	P		*C.albicans*	2	1	0.125	0.0313	
93	Rajan	65	M	Farmer	108977	DLSO	5	10	2	168	1	TN	P		*Trichophyton rubrum*	32	0.5	0.5		0.031
94	Janaki	49	F	Housewive	2840/14	PSO	5	7	2	130	1	FN	N		*NG*					
95	Mani	54	M	Farmer	8297	DLSO	4	20	2	115	1	TN	P	Post renal transplant patient	*Fusarium species*	32	32	8	4	
96	Sakar	66	M	Farmer	11209	DLSO	2months	4	2	142	1	FN	P		*NG*					
97	Rajan	54	M	Farmer	14208	DLSO	2	6	2	180	1	TN	P	T.pedis	*NG*					
98	Kumaran	59	M	Farmer	69724	DLSO	4	5	2	122	1	TN	P		*NG*					
99	Akthar	62	M	Watch man	110774	DLSO	5	4	2	168	1	TN	P		*C.tropicalis*	1	1	0.125	0.0313	
100	Veerammal	58	F	Housewive	3525	DLSO	4	6	2	260	2	TN	P	H/O trauma	*C.parapsilosis*	2	0.5	0.0625	0.0313	
101	Rani	64	F	Housewive	66110	DLSO	3	15	2	240	1	TN	N		*NG*					
102	Lakshmanan	65	M	Farmer	187214	DLSO	4	11	2	210	1	TN	P	H/O trauma	*Fusarium species*	64	32	8	8	
103	Nagarajan	54	M	Farmer	12810	DLSO	2	2	2	220	1	TN	P	H/O trauma	*Fusarium species*	32	32	16	4	
104	Manju	52	F	Farmer	6297/13	DLSO	3	2	2	168	2	TN	P		*Fusarium species*	64	32	16	4	
105	Vellammal	45	F	Housewive	36/14	DLSO	8	8	2	196	1	TN	P	H/o frequent hand washing	*C.parapsilosis*	1	0.5	0.0625	0.0313	
106	Rose	53	F	Housewive	9102	DLSO	2	1month	2	188	2	TN	P		*NG*					
107	Shantham	56	F	Shopkeeper	207784	TDO	8	18	2	162	10	TN	P	T.glutealis,T. corporis	*E.flocosum*	16	1	0.5		0.031
108	Dasan	61	M	Farmer	005/14	DLSO	4	10	2	192	2	TN	N		*NG*					
109	Nadarajan	58	M	Farmer	0011/2014	DLSO	6	3	2	148	1	TN	P		*NG*					
110	Vasuki	57	F	Farmer	185132	DLSO	8months	3	2	196	1	FN	P		*A.fumigatus*	32	0.5	0.5	0.0313	
111	Kumari	53	F	Housewive	007/2014	DLSO	3	4	2	126	2	TN	P	Hypothyroid ism	*NG*					

112	Sasi	28	M	Engineer	033/12	DLSO	2	newly detected	1	224	1	FN	N	T.cruris	*NG*				
113	Mari	56	F	Housewive	8297	DLSO	6	21	2	140	2	TN	N		*NG*				
114	Jagammal	49	F	Farmer	2940/14	DLSO	5	10	2	145	1	FN	P	T.cruris	*C.albicans*	2	0.5	0.0625	0.0313	
115	Rajavelu	64	M	Farmer	109962	DLSO	6	10	2	164	1	TN	P	T.pedis	*Trichophyton tonsurans*	8	0.5	0.25		0.031
116	Lakshmi	44	F	Housewive	61241	DLSO	4	4	2	146	1	FN	P		*C.parapsilosis*	1	1	0.125	0.0313	
117	Xavier	45	M	Watch man	312084	DLSO	4	5	2	144	1	TN	P		*NG*					
118	Rajalakshmi	61	F	Housewive	12641	DLSO	2months	16	2	174	1	TN	P	T.corporis	*NG*					
119	Mariammal	61	F	Farmer	14214	DLSO	10	12	2	124	1	FN	P		*C.parapsilosis*	2	1	0.0625	0.0313	
120	Devan	68	M	Farmer	105418	DLSO	8	14	2	120	1	FN	P	HT	*NG*					
121	Gopal	62	M	Farmer	54681	DLSO	9months	15	2	140	1	TN	P		*Fusarium species*	32	16	16	4	
122	Venilla	44	F	Housewive	187891	DLSO	4	2	2	160	1	TN	N		*Trichophyton rubrum*	32	2	0.5		0.031
123	Jersy	46	F	Housewive	12148	TDO	5	7	1	120	1	TN	N		*NG*					
124	Rahuman	66	M	Farmer	102787	DLSO	15	12	2	170	1	TN	P	HT	*Fusarium species*	32	32	8	4	
125	Kasinadan	62	M	Farmer	46812	DLSO	5	4	2	168	1	TN	P	HT	*NG*					
126	Mangai	46	F	Housewive	91461	TDO	8	7	2	124	20	Both	P		*NG*					
127	Aadi	46	M	Farmer	54196	DLSO	6months	1	2	210	1	TN	P		*NG*					
128	Mumtaj	32	F	Housewive	42564	DLSO	4	2	2	120	11	Both	P	HT	*C.parapsilosis*	2	0.5	0.0625	0.0313	
129	Maideen	55	M	Watch man	25241	DLSO	4	1	2	180	2	TN	P		*NG*					
130	Malar	50	F	Farmer	151421	DLSO	2	4	2	110	2	Both	P		*C.albicans*	2	1	0.125	0.0313	
131	Balan	45	M	Carpenter	74142	DLSO	2	3	2	140	2	FN	N		*NG*					
132	Rosy	48	F	Housewive	14208	DLSO	8	7	2	122	1	TN	P		*NG*					
133	Basu	52	M	Driver	38667	DLSO	1 1/2months	6months	2	124	20	Both	P	H/O trauma	*Fusarium species*	64	32	16	4	
134	Vasanthi	49	F	Housewive	42634	DLSO	6	1	2	162	2	TN	P	H/O trauma,T.corporis	*Trichophyton rubrum*	32	2	0.5		0.031
135	Parthiban	40	M	Farmer	62443	DLSO	6months	4	2	124	2	TN	N		*NG*					
136	Kadhir	42	M	Farmer	31674	DLSO	6months	4	2	148	1	FN	P	T.glutealis,T.corporis	*Trichophyton tonsurans*	8	0.5	0.25		0.031
137	Anbuselvi	46	F	Housewive	32546	DLSO	3months	4	2	150	6	TN	P	T.corporis ,Hypothyrodism	*NG*					
138	Mary	56	F	Housewive	14246	DLSO	4	1	2	400	2	TN	N		*NG*					
139	Kandasamy	40	M	Farmer	16421	DLSO	2	5	2	200	2	TN	P		*NG*					
140	Chandran	39	M	Farmer	56202	DLSO	3months	2	2	142	2	TN	P	HT	*C.parapsilosis*	1	0.5	0.0625	0.0313	
141	Lakshmi	52	F	Farmer	24612	DLSO	2	7	1	162	2	TN	P		*NG*					
142	Vairam	71	F	Housewive	12714	DLSO	1	16	2	128	2	TN	P	H/o trauma	*C.parapsilosis*	2	1	0.0625	0.0313	
143	Ravi	50	M	Farmer	16524	DLSO	20	2months	2	342	2	TN	P		*NG*					

144	Vasagan	63	M	Farmer	9311/10	DLSO	4	6	2	152	11	Both	P		*NG*					
145	Sindhamani	58	F	Farmer	9278/11	DLSO	8	7	2	138	1	TN	N		*NG*					
146	Abdul	55	M	Driver	46066	TDO	6months	6	2	128	11	Both	N		*NG*					
147	Grace	42	F	Housewive	51611	DLSO	5	4	2	145	1	FN	P		*C.albicans*	2	0.5	0.0625	0.0313	
148	Murugan	51	M	Farmer	2184	TDO	3	6months	2	176	2	TN	P		*NG*					
149	Ilakiya	42	F	Farmer	48518	DLSO	5months	6	2	180	2	FN	P		*A.niger*	4	1	0.5	0.0313	
150	Dhanam	47	F	Housewive	160118	DLSO	10	5	2	300	1	TN	P		*NG*					
151	Sundaram	46	M	Driver	12181	DLSO	2	5	1	160	2	Both	P	H/o trauma	*C.tropicalis*	2	1	0.125	0.0313	
152	Sivan	51	M	Farmer	14182	DLSO	4	6	2	180	1	TN	N		*NG*					
153	Maasi	55	M	Farmer	12185	TDO	4	6	2	145	1	TN	N	HT	*NG*					
154	Ragavan	62	M	Farmer	8542	DLSO	3	4	2	126	1	FN	P		*C.parapsilosis*	2	0.5	0.0625	0.0313	
155	Radhi	51	F	Staff nurse	189961	DLSO	3	4	2	148	1	FN	P	HT	*C.parapsilosis*	2	0.5	0.0625	0.0313	
156	Raniammal	48	F	Clerical staff	61421	DLSO	5	4	1	152	1	FN	P	T.cruris	*NG*					
157	Shanti	42	F	Housewive	12814	DLSO	2months	5	2	145	2	TN	P		*NG*					
158	Chinnamma	58	F	Farmer	15214	DLSO	4months	8	2	160	2	TN	P		*A. fumigatus*	16	0.5	0.5	0.0313	
159	Rani	51	F	Farmer	54871	DLSO	4	7	2	128	1	TN	P		*NG*					
160	Rakayee	60	F	Farmer	621812	DLSO	4months	12	2	140	1	FN	P	T.pedis	*NG*					
161	Velan	58	M	Farmer	51214	DLSO	6	10	2	180	2	TN	N		*NG*					
162	Rajammal	74	F	Housewive	12146	TDO	4	15	2	168	1	TN	N		*NG*					
163	Vadivel	48	F	Farmer	13214	TDO	1	13	2	158	1	TN	P		*NG*					
164	Kulandhai	56	M	Farmer	15124	DLSO	4months	6	2	128	2	TN	N		*NG*					
165	Velu	74	M	Shopkeeper	181433	DLSO	4	10	2	128	1	TN	P		*C.parapsilosis*	1	1	0.0625	0.0313	
166	Chandran	48	M	Farmer	1920/14	DLSO	5	1	2	164	10	TN	P	HT	*NG*					
167	Dhanam	51	F	Farmer	24113	DLSO	5	7	2	194	1	TN	P		*Fusarium species*	32	32	8	4	
168	Thilagam	47	F	Housewive	191412	DLSO	4	9	2	198	1	TN	N	Hypothyroidism	*NG*					
169	Sathya	39	F	Staff nurse	15143	TDO	4	4	1	120	1	TN	P		*NG*					
170	Rasu	74	M	Clerical staff	19143	DLSO	5	6	2	148	1	FN	P	T.corporis	*C.albicans*	2	1	0.125	0.0313	
171	Eswaran	78	M	Clerical staff	181421	DLSO	4	15	2	178	20	Both	N	T.corporis	*NG*					
172	Kodi	42	F	Farmer	0015/11	DLSO	5	3	2	180	1	TN	P		*C.tropicalis*	1	1	0.0625	0.0313	
173	Chellam	55	F	Farmer	181521	DLSO	8	8	2	152	2	TN	P		*NG*					
174	Subramani	54	M	Farmer	19821	DLSO	9	9	2	148	2	TN	N		*NG*					
175	Pandi	55	M	Farmer	0781/12	DLSO	6	7	2	160	2	TN	N		*NG*					

MASTER CHART – KEY

M	-	Male
F	-	Female
FN	-	Finger nail
TN	-	Toe nail
NG	-	No growth
KOH	-	Potassium hydroxide
P	-	Positive
N	-	Negative
DLSO	-	Distal Lateral Subungual Onychomycosis
PSO	-	Proximal Lateral Subungual Onychomycosis
TDO	-	Total dystrophic Onychomycosis
MIC	-	Minimum inhibitory concentration
DM	-	Diabetes mellitus
HT	-	Hypertension
CKD	-	Chronic Kidney Disease
FLU	-	Fluconazole
ITRA	-	Itraconazole
KET	-	Ketoconazole
VORI	-	Voriconazole
TERBI	-	Terbinafine

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